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13. ABSTRACT (Maximum 200 Words) In the first year of this study, we have developed gene therapy approaches for fracture repair by: 1) identifying regulatory sequences providing maximum transgene expression by two gene therapy vectors in the bone cells that mediate fracture repair; 2) developing vector delivery techniques for optimal therapeutic gene expression in the healing fracture; and 3) identifying therapeutic gene candidates to augment and accelerate fracture repair. Maximum transgene expression in primary and transformed bone cells was obtained using the non-specific murine leukemia (MLV)-based long terminal repeat and the lentiviral cytomegalovirus promoters, and the gene-specific elongation factor 1α promoter with the lentiviral-based vector. Using the MLV-based vector, surgical techniques have been developed to deliver gene therapy to the fracture site and optimize transgene expression for optimal therapeutic effect. These techniques have benefited healing by augmenting BMP-4 mediated bone formation in the fracture gap. The healing fracture tissues of four individual subjects have been examined for gene expression by microarray analysis at both 3 days and 11 days healing to identify therapeutic gene candidates. Several hundred genes and expressed sequence tags displayed statistically significant changes in expression at each time. Several candidates are currently undergoing confirmation of expression and further functional characterization.						
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INTRODUCTION

Battlefield injuries to the musculoskeletal system optimally require rapid wound healing and occasionally regeneration of the wounded tissues. Gene therapy has great potential to help rapidly heal such battlefield injuries, including soft-tissue as well as hard-tissue injuries. Fracture repair requires a complex series of molecular events that coordinate the proliferation and differentiation of diverse periosteal tissues that bridge the injury with bone that is identical to native bone and lacks scar tissue (Bolander, 1992). Different families of growth factors have been implicated that mediate diverse pathways of cell chemotaxis and tissue proliferation and differentiation during fracture repair (Barnes et al., 1999; Beasley and Einhorn, 2000). To elucidate the molecular pathways that mediate repair of diverse fracture tissues, the expression of growth factors and their receptors during fracture repair must be characterized and the physiological and morphological effects following local or systemic administration of their genes must be tested. However, gene therapy technology is still in its infancy and scientists have only begun to develop systems that both deliver growth factor genes and regulate their expression in injured tissues to augment normal and impaired healing.

Currently, our gene therapy approach has the potential to regenerate large skeletal defects, certainly an advantage for the repair of battlefield injuries of the musculoskeletal system. In our studies, we are optimizing fracture gene therapy using the hybrid growth factor transgene, bone morphogenetic protein (BMP)-2/4, expressed from constitutively by a Murine leukemia (MLV)-based vector or in a gene-specific manner by a lentiviral-based vector. Surgical techniques have been developed to optimize the delivery of the viral vector of choice to the healing fracture in small animals. The combination of efficient vector expression with optimal surgical delivery to the fracture will maximize the therapeutic effect for study. However, we also anticipate that to truly optimize gene therapy for fracture healing, more than one therapeutic gene may be required. In light of this, we have undertaken microarray studies of the fracture callus of multiple individual animal subjects to understand both gene expression in the response to bone injury, as well as those molecular factors that might delay the healing of such injuries. Several hundred genes that exhibit changes in expression at two different healing times have been identified, and those with therapeutic potential will be considered for inclusion in study in our gene therapy system. We propose that the efficient delivery and expression of growth factor, receptor and corresponding signaling pathway genes will, when expressed with BMP-4, enhance fracture healing. Ultimately, the development of these approaches could not only enhance the broad-spectrum fracture repair, but genetic algorithms to predict the response of individual military personnel to battlefield injury could individualize their therapy.

Our goals for the first twelve months of the funding period for each of the Technical Objectives below, as well as progress to date for each technical objective, are described below. This progress report is organized according to the outline provided by the office of the US Army Medical Research and Material Command.

In addition to completing all the Technical Objectives required for this report period, we are reporting progress on additional objectives accomplished above and beyond those contracted for. These additional objectives are identified in the report as they are pertinent.

BODY

1. TECHNICAL OBJECTIVE 1: TO OPTIMIZE A GENE THERAPY FOR FRACTURE HEALING
 - a) Specific Objective 1: To Optimize Gene Expression and Protein Production of Growth Factor genes from Periosteal Cells Transduced with MLV-based or Lentiviral-based Vectors

- 1) Objective

This goal of this study is to identify the optimum viral-based vector and regulatory (promoter) elements for fracture gene therapy by comparing transfection and expression frequencies of Murine Leukemia virus (MLV)-based and lentiviral-based gene therapy vectors and promoters in periosteal and endosteal cells.

- 2) Materials and Methods

Periosteal cells were aseptically isolated from the unfractured hindlimb bones of Sprague-Dawley rats. The femurs and tibias were removed at sacrifice, the epiphyses removed and the diaphyseal marrow ablated with saline. The diaphyses were digested once with 0.25% trypsin and twice with 0.20% collagenase II for 2 hours each. The digests separated the periosteal and endosteal cells from the cortical bone and yielded dispersed and heterogeneous populations of the fibroblastic and cambial cells. These adherent cells were retained in culture while nonadherent contaminating marrow cells were discarded. Cell preparations of periosteal and endosteal origins provided a representative population of cells to be targeted for viral transduction by external or internal injection techniques. These cells were cultured at a low passage number for the subsequent comparison of transgene expression from transfected MLV-based or the lentiviral-based vectors.

To obtain optimal estimates of the relative transgene expression efficiencies for the MLV-based and lentiviral-based vectors, the Enhanced Green Fluorescent Protein (EGFP) was used as the transgene. Vector efficiency was established by target cell EGFP expression, which was regulated by the long terminal repeat (LTR) promoter in the case of the MLV-based vector, or by one of five gene-specific promoters in the case of the lentiviral-based vector. These five gene-specific promoters were derived from the cytomegalovirus (CMV), elongation factor 1-alpha (EF-1a), collagen 1, collagen 2.3 and core-binding factor alpha-1 (cbfa-1) genes.

Each vector and promoter-EGFP combination was transduced into the periosteal and endosteal cell cultures in duplicate ($n = 2$). Additionally, the mouse osteoblast MC-3T3 cell line was assayed for EGFP expression as a known bone cell line. Because variable transgene expression from different promoters makes the virus difficult to titer, 10 μ l and 50 μ l volumes of each viral vector stock were compared for transfection and expression frequencies. The numbers of cells expressing EGFP were quantified by fluorescent cell sorting following 48 hours of culture.

3) Results

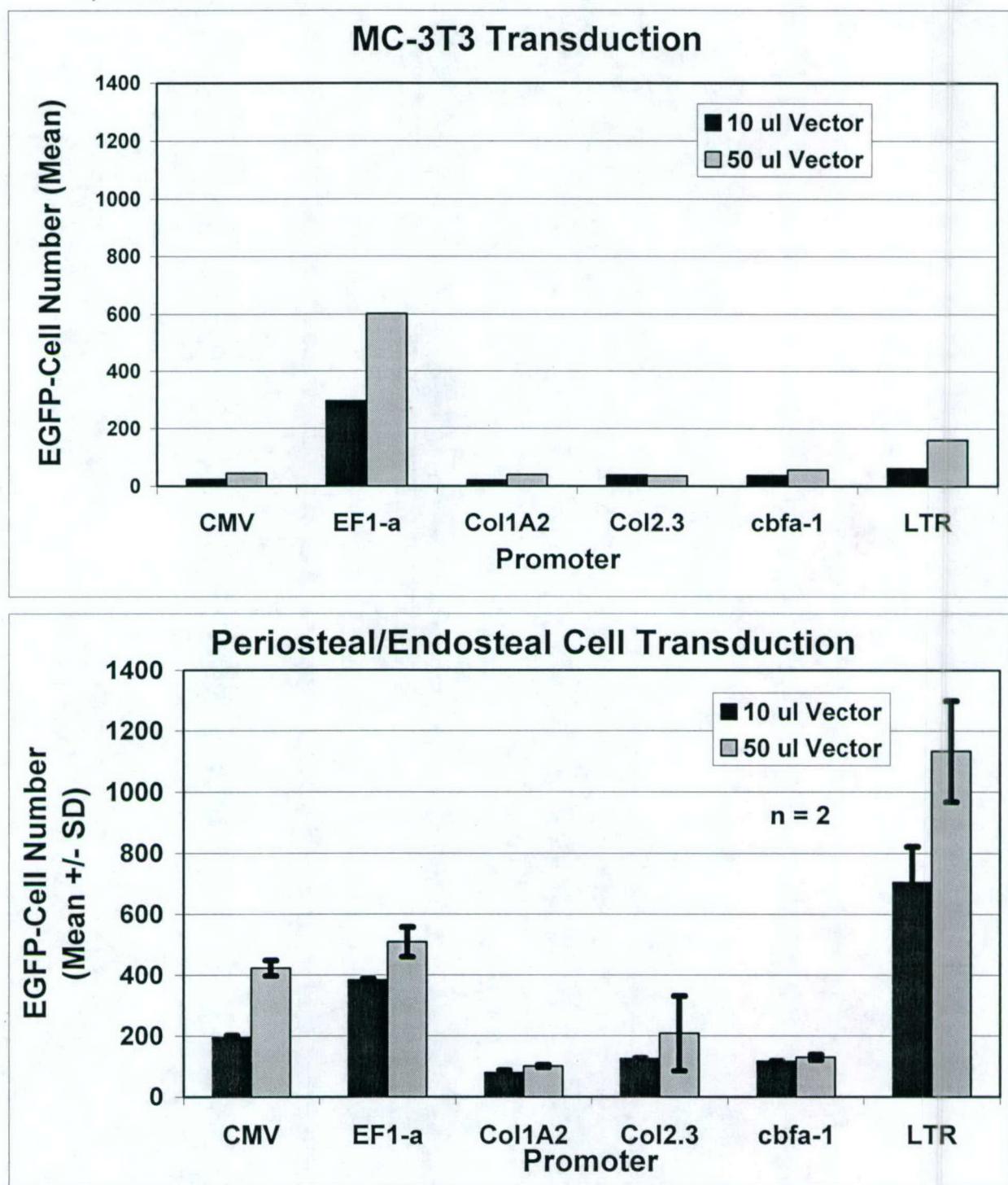


Figure 1. Mean EGFP expression in MC-3T3 (top) periosteal/endosteal bone cells (bottom) from the LTR promoter in an MLV vector, or other promoters (CMV, EF1-a, Col IA2, Col 2.3, cbfa-1) in a lentiviral-based vector.

As expected, the powerful LTR promoter of the MLV-based vector provided the greatest numbers of EGFP-expressing cells in the endosteal/periosteal cells (Figure 1). Among the lentiviral-based vector promoters, the EF-1a promoter provided the best expression at the 10 ul transfections, with more cells expressing the transgene than even the very robust CMV promoter. Surprisingly, the promoters derived from genes normally associated with bone cells, but most notably the collagen 2.3 and the cbfa-1 displayed much weaker transfection and expression frequencies. The results were consistent at both volumes of viral vectors transfected, although there was a more pronounced improvement in the MLV-based vector and the lentiviral-CMV promoter combination at the 50 ul volume than with the other lentiviral-based vector promoters. The lentiviral-EF1-a promoter results were also observed in the MC-3T3 cell line, suggesting that the results in the isolated periosteal and endosteal cells were indeed representative of bone cells. In this case the expression from this promoter was even greater than the MLV-LTR.

4) Conclusions

We conclude that either the MLV-based vector with the LTR promoter or the lentiviral-based vector with the EF-1a promoter provide the optimal transfection and expression of the transgene in the bone cells expected to modulate fracture healing. These vector and promoter combinations will be used for therapy when the optimal delivery technique (Specific Objective 2, below) and the therapeutic gene candidate(s) (Technical Objective 2, microarray analysis) are identified.

b) Specific Objective 2: To Compare the Local Periosteal Injection of the Virus at the Fracture Site with the Intramedullary Inoculation Procedure

1) Objective

Surgical techniques have been adapted to apply the viral-based vectors from either the exterior aspects of the rat femoral fracture callus or through the intramedullary space to the interior of the fracture callus. Multiple exterior injections and intramedullary injection techniques have been developed to maximize the symmetry localization of growth factor expression to the fracture site and compared for their ability to maximize the therapeutic benefit of the growth factor. We hypothesized that the medullary pressure injection would provide a more symmetric distribution of the therapy to the fracture.

2) Materials and Methods

i) *Fracture Surgery*

The fracture surgery for exterior injections is as previously described in the rat femur fracture model (Bonnerans and Einhorn, 1984). A stainless steel Kirschner wire (pin) is inserted into the femoral medullary canal to stabilize the fracture (Figure 2a), which is produced immediately after surgery by the three-point bending technique (Figure 2c). Post-fracture injections are performed from the exterior lateral or medial aspects of the leg. However, we have also adapted this surgery to aseptically insert a 20G catheter into the medullary canal of the femur alongside the stabilizing pin (Figure 2b, 2c). This catheter permits the post-fracture anterograde insertion of a needle into the medullary canal from the greater trochanter for the delivery of vectors expressing growth factor genes to the interior of the fracture at different times after the fracture.

ii) *Fracture Injection*

The therapeutic gene chosen was the BMP-2/4 hybrid gene and the B-galactosidase gene was chosen as the control (marker). Each was expressed from the MLV-based vector (Figure 3).

Usual Fracture Surgery



A

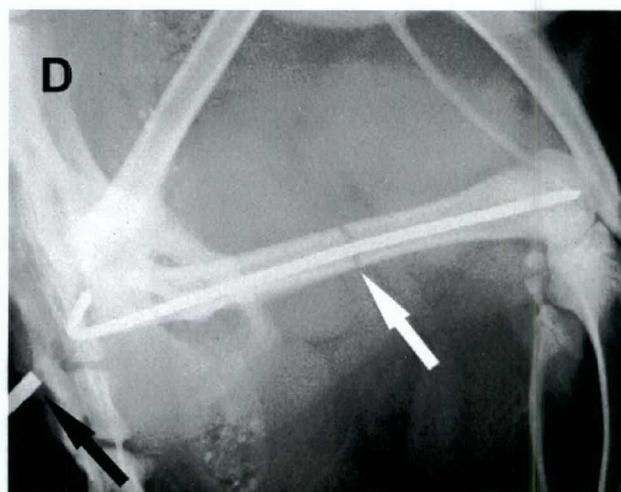
Catheter Modification



B



C



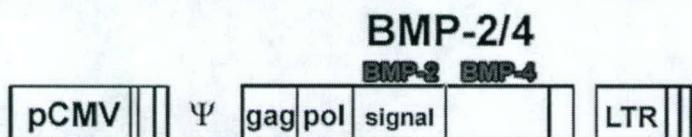
D

Figure 2. The usual femur fracture surgery (left) and catheter modification (right).

A stabilizing Kirschner wire is inserted into the medullary space, hooked (A), seated at the greater trochanter and cut flush with the condyle prior to fracture (C, white arrow) by three-point bending.

In the catheter insertion modification, a 20 gauge catheter is inserted from the greater trochanter beside the wire inside the medullary space (B). The fracture is again produced by three-point bending technique (D, white arrow). The external catheter hub is visible (D, black arrow), though the internal tubing cannot be visualized by X-Ray.

pCLSABMP-2/4 (therapeutic gene)



pCLSAB-gal (marker control gene)



Figure 3. MLV gene constructs used for injection into the fracture.

The hybrid BMP-2/4 transgene (top) contains the human BMP-4 gene and BMP-2 signal sequence. The signal sequence was found to enhance BMP-4 secretion. The bacterial B-galactosidase gene has previously served both as a marker gene and as a non-therapeutic control gene for comparison with the BMP-2/4 gene. Expression for each gene is driven by the long terminal repeat (LTR) of the MLV-based vector.

We have previously used the fracture model for exterior injections of these genes to the lateral aspect of the femur (Figure 4a; Rundle et al., 2003). These injections attempt to deposit the therapy in the periosteal cambial layer on the cortical surface of the fractured femur. The accuracy of these injections was maximized using a fluoroscope to visualize the injection point relative to the fracture (Figure 4c). The fluoroscope was also used to monitor the accuracy of injections through the intramedullary catheter. The needle was easily inserted through the external opening of the catheter (Figure 4b), into the medullary space, and placed immediately proximal to the fracture for the injection (Figure 4d). In each case, 150 ul of vector was injected, a volume calculated to deliver between 15 million and 150 million viral particles at the titers produced by our Viral Vector Core Facility.

Because we have been testing these applications with the MLV-based vector, which requires proliferating tissue targets for transduction, we have injected the fracture tissues at one day post-fracture, when cell proliferation in the wound has started. However, because the lentiviral vector does not require actively proliferating cells for transduction, the post-fracture injection times can be altered to maximize the therapeutic benefit of a particular transgene.

3) Results

i) Marker Localization in the Fracture Following Exterior or Intramedullary Injection

Initially, we sought to localize fluids in the fracture by each injection method. To visualize the injection, 100 ul of a radio-opaque medical contrast dye was injected into the fracture site by either the lateral method or through the catheter. Its location was easily determined in real time using the fluoroscope. The injection of the contrast dye from the lateral aspect of the fracture was distributed in the muscle within the leg; very little appeared to penetrate the periosteum, where the target cells for fracture therapy reside (Figure 5a). The injection through the catheter was much more encouraging (Figure 5b). In this case, the contrast

Exterior (Lateral) Injection



Catheter Injection



Figure 4. Exterior (left) and interior (catheter, right) injection techniques.
Injections from the exterior lateral aspect of the leg (A) used a fluoroscope to accurately place the needle at the fracture periosteum for injection (C).
Catheter injections inserted a 25 gauge spinal needle through the catheter hub (B) into the femoral medullary space to deposit the injection at the fracture, again visualized using a fluoroscope (D).

dye filled the medullary space and leaked out the wounds at the (proximal) greater trochanter and the (distal) condyle, where the pin was inserted. However, much of the dye also appeared to distribute to the fracture. Based upon these results the volume of the medullary canal was estimated to be approximately 150 ul, and the accuracy of transgene delivery and efficiency of transgene expression was examined.

To compare the accuracy of transgene delivery and expression between exterior lateral and the catheter injection techniques, 150 ul of MLV-based vector expressing a B-galactosidase marker gene was then applied at one day post-fracture. The animal was sacrificed at one week of healing and B-galactosidase gene expression was localized by splitting the bone and staining both the exterior (Figure 5c) and interior (Figure 5d) for B-galactosidase activity. When applied from the exterior lateral aspect of the fracture, marker gene transfection and expression was confined to a variable area at the periosteal surface that is certainly of limited efficacy for the distribution of any therapeutic gene to the fracture periosteal target cells (Figure 5c and d, top). This injection technique is inherently difficult during early healing; although it is the most clinically relevant time for therapy, the periosteal tissue layer is very thin, and presents a poor target. Consequently, most of the injection must have spilled into the surrounding muscle, as observed with the contrast dye injection. However, when applied from the interior of the fracture at one day using an intramedullary catheter, B-galactosidase marker gene expression after the same healing period was more symmetrically distributed around the fracture circumference, propagating throughout the periosteal tissues (Figure 5c and d, bottom). In accordance with MLV-based vector requirements for proliferating cells for transduction, B-galactosidase expression remained localized to the wounded and proliferating tissues at the fracture site. The therapy was concentrated at the fracture, either not being retained in the medullary canal or, if retained, lacking the proliferating target cells necessary for transduction. The catheter surgery technique therefore successfully avoided creating intramedullary injury and resident cell proliferation substantial enough for MLV-based vector transgene expression, and successfully confined transgene expression to the injured tissues of interest, namely the fracture. Moreover, as we have previously observed, the fractures that received B-galactosidase gene displayed normal callus size and content. Expression of this marker gene can therefore be used as a control for comparison with therapeutic gene expression. In summary, the catheter application technique will be much better for the even distribution of prospective therapeutic genes and for the determination of their therapeutic value during healing.

ii) BMP-2/4 Gene Fracture Therapy Following Injection from the Exterior or Through the Intramedullary Catheter

Exterior injections of MLV-based vector expressing the BMP-2/4 transgene, either from the lateral aspect or a combination of lateral and medial aspects were compared with injections to the intramedullary space through the surgically implanted catheter. The BMP-2/4 gene was chosen as the transgene to develop the therapeutic delivery because of its documented ability to efficiently differentiate osteogenic precursors to bone (Peng et al., 2001). As such, inaccurate delivery of the transgene can be easily established by the appearance of ectopic bone.

The therapy produced by each injection was monitored at 7, 14, 21 and 28 days healing via X-ray examination for mineralized tissues in live animals. Lateral injections always produced asymmetric bone formation (Figure 6a, c, e, g) that was obviously ectopic at 14 and 21 days healing (Figure 6c, e), when endochondral bone formation is at its peak. This condition was almost certainly produced by transgene expression in interstitial muscle cells of the fractured leg

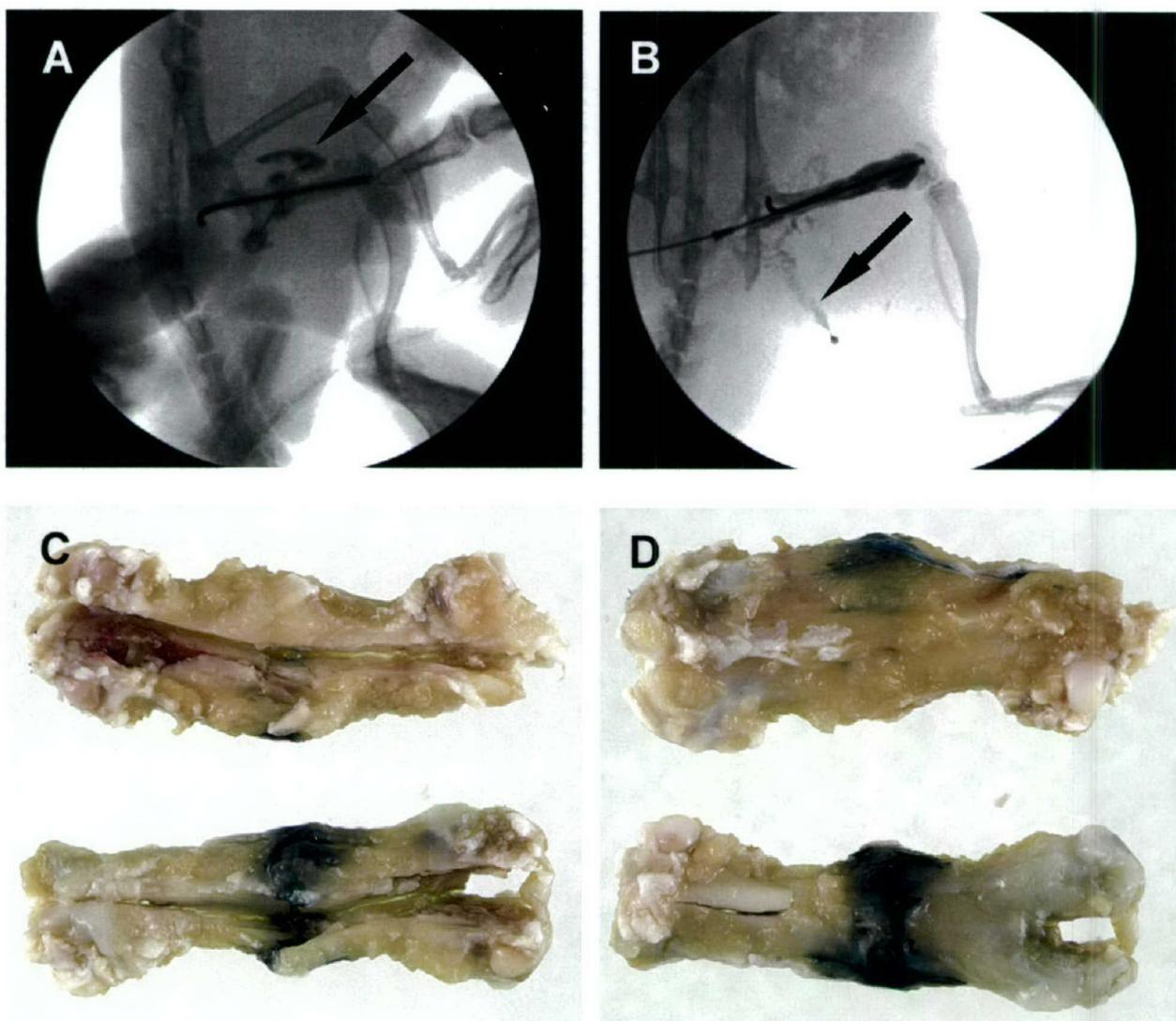


Figure 5. Marker localization in fractures injected from the exterior (lateral) aspect or through the catheter.

TOP: A fluoroscope was used to visualize a radio-opaque contrast dye during a lateral injection (A) or an intramedullary catheter injection (B). Despite best efforts, the dye immediately distributed in the leg muscles when injected from the exterior (A, arrow). When injected through the catheter, the dye was retained in the medullary space (B), except for some leakage visible at the greater trochanter a result of normal marrow fluid displacement (arrow).

BOTTOM: The MLV-based vector expressing the B-galactosidase marker transgene was injected at one day post-fracture and the femurs harvested at 7 days post-fracture, split open and stained for marker expression. The catheter injection (C, D, bottom) produced more extensive and symmetric marker expression in the interior (C) and exterior (D) of the fracture than the lateral injection (C, D, top).

that had been injured by the fracture technique and induced to proliferation. Such proliferation rendered them susceptible to MLV-based vector transduction of the BMP-2/4 gene that subsequently differentiated them to cartilage and bone. Although we have determined that this ectopic bone eventually remodels, the therapeutic benefits of tissues outside the periosteum, where fracture healing is normally mediated, are questionable. In contrast, this problem was avoided when the vector was injected through the catheter into the intramedullary space at the fracture site. In this case the bony tissues that developed were not only confined to subperiosteal tissues, but also filled the fracture gap at all times during healing (Figure 6b, d, f, h). The bony tissues appeared after one week of healing, the time when BMP-4 normally differentiates soft callus to bone, suggesting that the temporal BMP-4 gene expression was normal. Higher resolution X-ray and histologic examination of the fracture tissues of individual bones at 14 days (Figure 7a, b) and 28 days (Figure 7c, d) revealed that the fracture tissues consisted of normally appearing chondrocytes and osteoid characteristic of fracture repair. The tissue content of the fracture callus was normal, although this augmented tissue was ectopic in the lateral injections (Figure 7a, c) but subperiosteal and normal in the intramedullary catheter injections (Figure 7b, d). The latter method of therapy delivery, therefore, appears to provide symmetric transgene expression localized to the fracture and represents the greatest potential for measuring therapeutic effects of gene therapy. We will confirm BMP-4 gene expression in these tissues by immunohistochemistry.

Comparative analysis of the fracture tissues produced by exterior and intramedullary injections by peripheral quantitative computed tomography (pQCT) was not possible because of the inaccurate exterior injections resulted in large and variable amounts of ectopic bone spread throughout the muscle (Figure 7). However, a torsional method for the mechanical testing of bone strength in response to our gene therapy has been developed (Figure 8). Preliminary examinations have compared a fracture injected with the B-galactosidase control marker gene at 32 days healing with the unfractured contralateral femur from the same animal and established the conditions for torsional testing of our gene therapy. Our therapy will attempt to accelerate the return of the fractured bone to the load capacity and stiffness of an unfractured bone.

Exterior (Lateral) Injection



C



E



G



Interior (Catheter) Injection



D



F

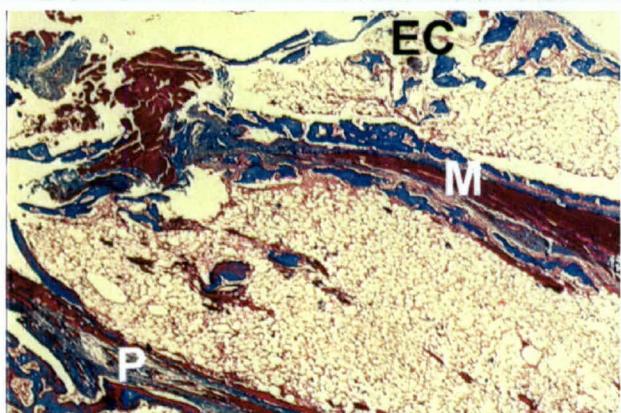
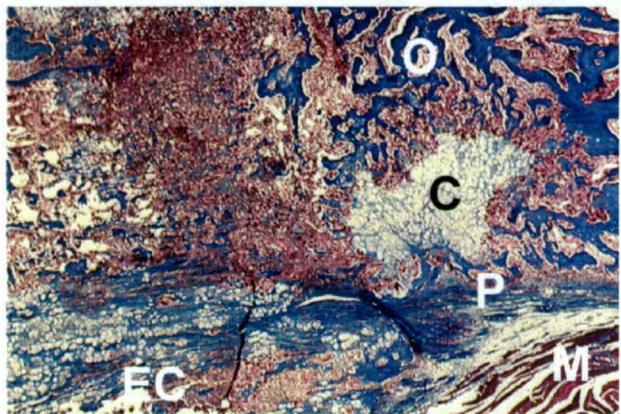


H



Figure 6. X-Ray Comparison of Mineralized Tissue During Healing.
Injection was performed from the exterior (left) or through the catheter and monitored at 7 days (A,B), 14 days (C,D), 21 days (E,F) and 28 days (G,H) healing. These X-Rays present two different individuals for each injection technique.

Lateral Injection



Catheter Injection

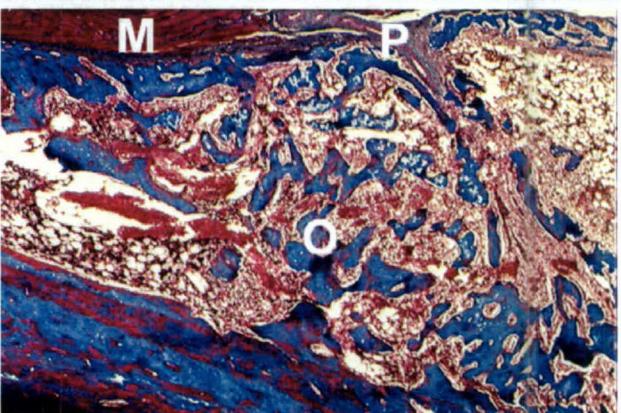
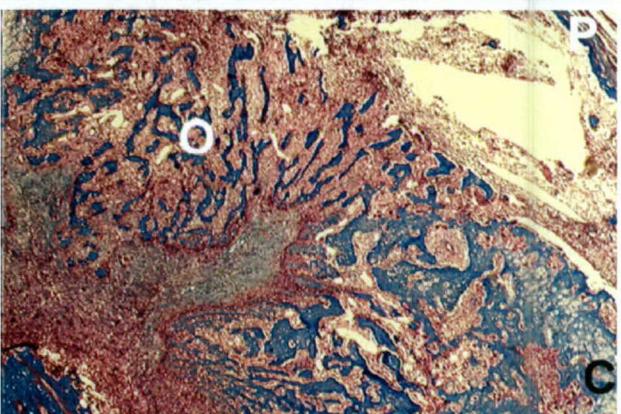


Figure 7. X-Ray and histology of fracture callus following lateral injection (left) or catheter injection (right) at 14 days (A, B) and 28 days (C, D) healing. Trichrome stains of the fracture callus revealed ectopic (EC) bone formation outside the periosteum (P) at 14 and 28 days in post-fracture lateral injections. Bone formation was under the periosteum (P), at 14 and 28 days in post-fracture catheter injections and did not involve the muscle (M). The fracture tissues appeared normal at all healing times. Cartilage (C), Osteoid (O).

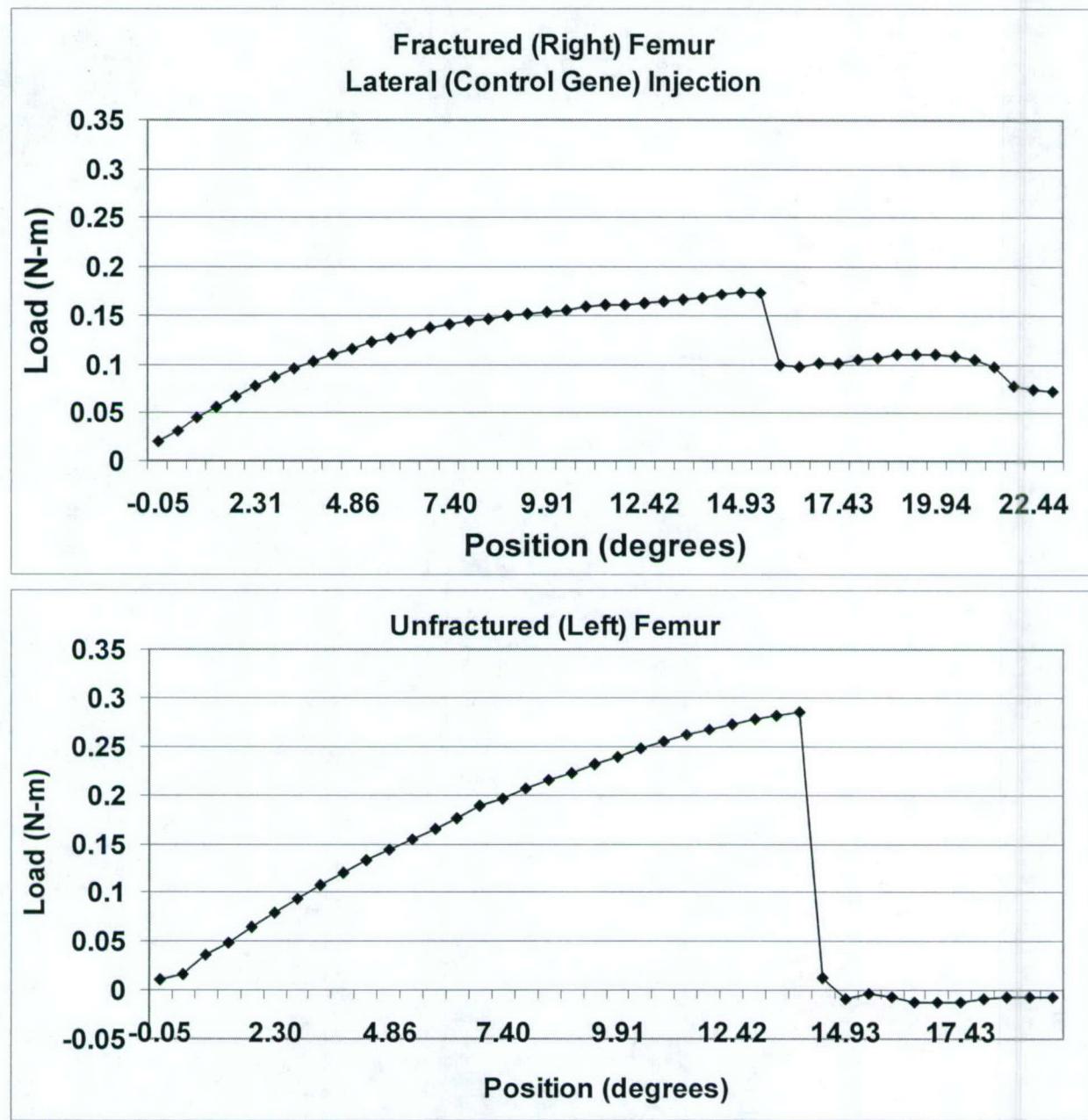


Figure 8. Illustration of the mechanical testing approach for measuring bone strength. A sample comparison of torsional testing conducted on a rat femur fracture injected with the B-galactosidase (nontherapeutic marker) gene from the lateral aspect and allowed to heal for 32 days (top) as compared with the unfractured contralateral femur from the same animal (bottom). At this time, which is approximately one week prior to bony bridging of the fracture gap, the fracture fails at a slightly larger torsional angle (15 degrees vs. 13 degrees) and approximately 2/3 load (in Newton-meters) of the unfractured femur. These parameters indicate reduced bone stiffness, calculated from the slope of the load vs. angle, and strength, determined by the ultimate load at failure. Such an application of torsional mechanical testing will establish augmentation in response to our gene therapy: the objective is the accelerated return of the fracture torsional strength and stiffness characteristics to that of an unfractured bone.

4) Conclusions

Analysis of injections from the exterior lateral aspect of the fracture demonstrated that extra-periosteal tissues were transduced with the BMP-2/4 transgene, and produced a large and asymmetric mineralized tissue that remained largely outside the periosteal boundaries. In these locations the bony tissues produced by the growth factor could not participate in bony union of the fracture gap, severely limiting the effectiveness of the gene therapy. Intramedullary injection of the MLV vector produced symmetric distribution of transgene expression confined to the periosteal tissues that appeared to augment bony tissues in the fracture gap. As such, this approach maximizes the potential of any therapeutic gene. These results are very conclusive, and we will pursue this technique of vector delivery to determine the efficacy of our different therapeutic genes for fracture healing.

c) Specific Objective 3: To Compare the Superiority of the MLV-based Versus the Lentiviral-based Vector Systems for the BMP-2/4 Transgene

1) Objective

This study will compare the efficacy of the lentiviral and MLV-based vectors for fracture healing. Each will express the BMP-2/4 transgene following delivery using the intramedullary catheter injection. The vector system that exhibits the greatest therapeutic benefits, as determined by bone formation and a return to prefracture mechanical strength, will be used in subsequent combination therapy with multiple transgenes. This study will begin shortly, as the surgical techniques for the vector delivery have just been developed.

d) Specific Objective 4: To Compare the Efficacy of the BMP-2/4 Transgene in the Optimized Vector System with that of the Combination of BMP-2/4 Transgene Plus Another Growth Factor Candidate Gene Identified by Micro-array (see Technical Objective 2)

1) Objective

This study will utilize the gene expression data in the normally healing fracture. *In addition to the microarray analysis of gene expression at 3 days healing originally proposed in Technical Objective 2, Specific Objective 2, we are also analyzing gene expression at 11 days healing.* This approach will provide gene candidates for both early fracture repair and later fracture repair. Work on this study awaits completion of the comprehensive analysis of the microarray gene expression data at these 2 time points. As pathways of gene expression are identified in fracture repair, one or more candidate genes will be selected for combination therapy with the BMP-2/4 transgene.

2. TECHNICAL OBJECTIVE 2: TO APPLY MICROARRAY TO STUDY FRACTURE HEALING

a) Specific Objective 1: To Extend the Number of Genes in Our Current In-house Micro-array Procedure

1) Objective

This study will identify and adapt the experimental microarray chip and analysis system best suited for the analysis of rat fracture gene expression.

2) Materials and Methods

We have adopted the Agilent rat oligomer chip to analyze our fracture RNA in our microarray analysis. This chip has 20,046 gene targets as 60-base oligomers and as such provides a more extensive number of rat gene targets than we are able to achieve with our facilities. Several major families of growth factors, signaling molecules and structural genes are represented, providing one of the most comprehensive surveys of rat gene expression currently available commercially. Most importantly, the "low-input" Agilent dye labeling system allows us to amplify the signal during fluorescent labeling of the cDNA. This approach is highly advantageous for reducing the RNA input into the system, minimizing the numbers of animals used yet maximizing the sensitivity of the microarray analysis for samples with very low amounts of tissues. This is particularly important in the unfractured but pinned control samples of the femur fracture model, which have very little tissue. This labeling system permits us to perform the analysis on these extremely limited samples without pooling the RNA from multiple individuals. We are therefore able to analyze the biological variation between subjects not possible in pooled samples, a problem often ignored in microarray experiments. The Agilent "low input" dye labeling technique was compared with the TSA amplification technique that we have used previously, and with analysis using no amplification. Using 2 ug of RNA for Cy3 and Cy5 dye-labeling, we compared the images following hybridization to the Agilent rat gene chip (below).

3) Results

Below (Figure 9) are scatter plots of the universal RNA labeled with the Agilent amplification protocol and the tyramide signal amplification (TSA) amplification protocol. The data was normalized identically between the arrays as follows, since a Lowess normalization cannot be performed with less than 1000 spots. The plots should give tight clustering around a slope of one since the same universal RNA was labeled using the respective protocols and Cy3 and Cy5 prior to hybridization. For the Agilent low input labeling method, the linear RNA amplification does not introduce artifacts. The only data points lying outside the 2 fold lines are the ratio spike in controls and these follow the expected ratios fairly well (top). The TSA plot (bottom) does not follow the expected slope of one well and the spike in ratio controls are not as consistent as with the Agilent labeling. Each gene's measured intensity was divided by its control channel value in each sample; if the control channel was below 10 then 10 was used instead. If the control channel and the signal channel were both below 10 then no data was reported. All of the genes in each sample were divided by the median of a user-specified list of positive control genes. The median of the positive control genes was calculated using only raw measurements above 10. Of the genes in the positive control list, only genes marked present were used. Positive control genes for normalization included the housekeeping genes glyceraldehydes phosphate dehydrogenase and beta-actin.

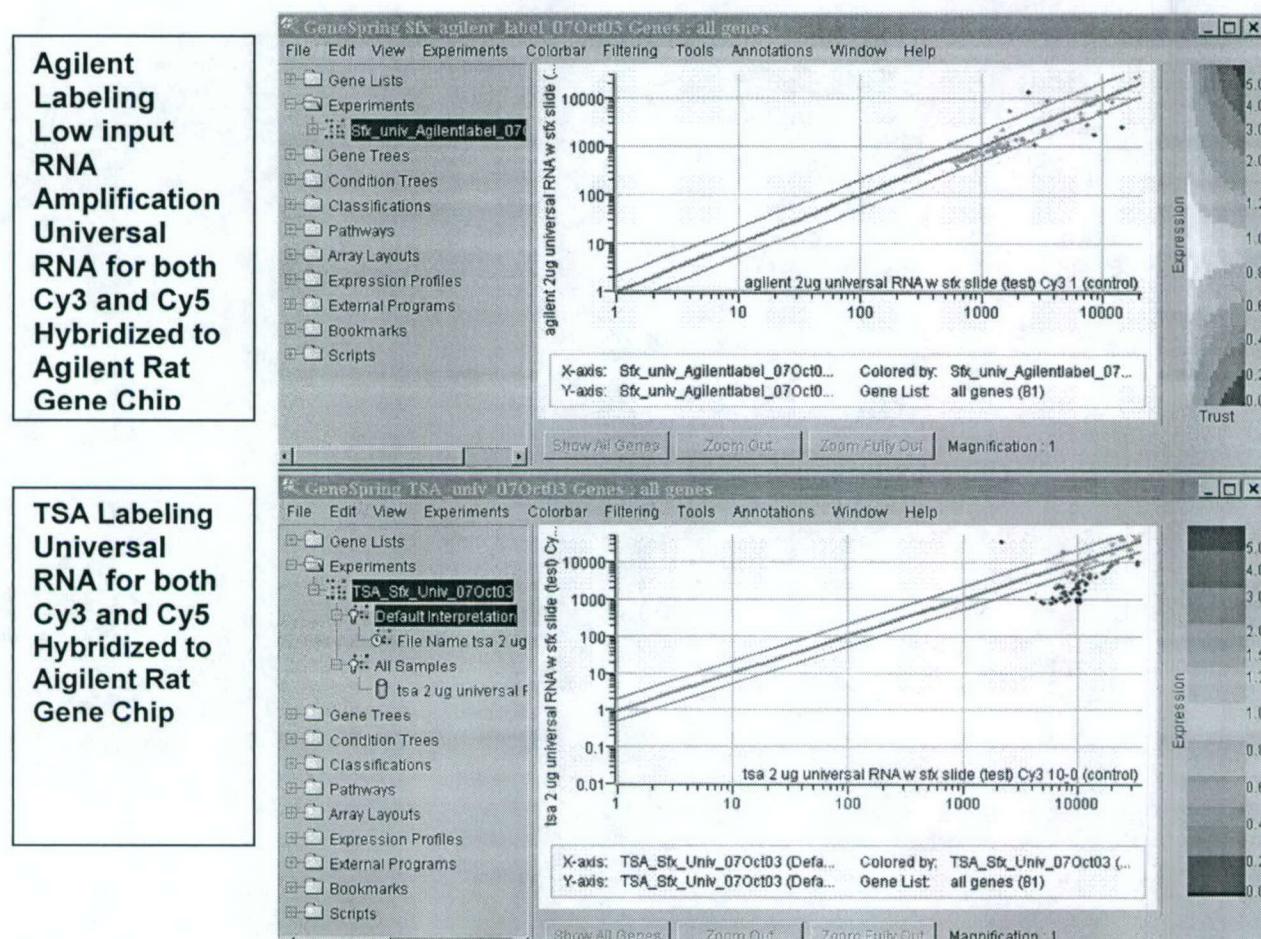


Figure 9. Scatter plot comparison of the Agilent “low input” (top) and TSA (bottom) dye labeling hybridization results on the Agilent rat gene chip.

4) Conclusions

The Agilent “low input” labeling system provides the most sensitive and accurate cDNA labeling system available, and provided superior hybridization images for reproducible analysis of gene expression. We will utilize the Agilent rat gene chip and dye labeling system for our fracture microarray analysis.

b) Specific Objective 2: To Apply Our Extended In-house Microarray to Study Gene Expression in Fracture Callus at 3 Days After Fracture

1) Objective

This study will identify therapeutic gene candidates for fracture gene therapy. Tissues have been harvested for analysis at two time points in healing: 3 days and 11 days. The early time is characteristic of the transition of the inflammatory phase to intramembranous bone formation and was the stated goal for this objective, but we have also included the latter time point, which is characteristic of the maturation of the cartilage intermediate to endochondral bone. Each represents a critical time when fracture repair genes should be expressed and identified in our microarray; the early time point will suggest gene candidates for early clinical

intervention, and the addition of the 11-day data should provide a more comprehensive measurement of gene expression during fracture healing.

2) Materials and Methods

Fracture surgery was performed as described above in Technical Objective 1, Specific Objective 2 (Materials and Methods, Fracture Surgery). The stabilizing pin was inserted and the femur fractured by three-point bending. No catheter was inserted. The control animal femurs were stabilized with an intramedullary pin but not fractured. Because fracture histology in previous studies had identified bone formation around the stabilizing Kirschner wire, this approach controlled for the effects of the stabilization on the healing process.

Femur fracture surgery was performed and RNA isolated from 4 individual fractured femurs at 3 days and 4 individual fractures at 11 days healing, with the fractures compared to equal numbers of individual unfractured (control) femurs at each time point. RNA isolation was performed on pulverized fracture tissues by guanidinium isothiocyanate and phenol extraction (Nemeth et al., 1989). The Cy3 and Cy5 labeling was performed as described in the Agilent "low input" labeling system, and the hybridization performed using equipment and procedures specified in the Agilent 20,046 rat gene chip. We compared each group of fractured RNA and unfractured (but stabilized) control RNA isolates at each time point, 3 days or 11 days post-fracture. Because the Agilent RNA dye labeling system allowed us to analyze fracture and nonfracture gene expression in individual animals, pooling of individual samples was avoided.

Microarray image analysis was also performed in-house, using ScanArray image analysis and Genespring expression analysis software. Lowess normalization was performed to identify differences in the Cy3 or Cy5 dye labeling efficiencies. One-way analysis of variance (ANOVA) established significant changes in expression of up-regulated genes and down-regulated genes for each group of fractured (as compared to unfractured (control)) animals at: 1) 3 days healing; 2) 11 days healing; and 3) additionally, these changes were used to identify combined significant changes in gene expression among all fractured versus nonfractured individuals at 3 days versus 11 days healing.

3) Results

All animals appeared healthy and comfortable, and were mobile at 1 day post-fracture. All fractures were examined at surgery and were midshaft and transverse. All fractures were also examined at sacrifice for evidence of fibrosis due to irritation or migration of the stabilizing pin. None was observed and the fracture callus appeared normal at each harvest time.

The total RNA recovery was routinely 20 ug to 30 ug from fracture tissues and 3 ug to 10 ug from unfractured controls; which had much less tissue than the fractured bone and therefore yielded much less RNA. The bone marrow, another source of nonfracture gene expression, was partially ablated in all animals by removing the pin upon dissection. Spectrophotometric and agarose gel analysis confirmed that the RNA had excellent purity and integrity, respectively.

Venn diagrams (Figures 10, 11 and 12) provide a graphical representation of the statistical analysis of gene expression at 3 days, 11 days, and combined 3 and 11 days healing. Tables 10, 11 and 12, respectively, list the genes that were significantly more than 2-fold up or down-regulated at each of these healing times.

1. At 3 days healing, of the 757 genes with expression differences determined to be significant by ANOVA, 754 genes were more than 2-fold up-regulated, and 3 genes were

Figure 10: Venn Diagram Illustrating Statistical Analysis of Fracture Gene Expression at 3 Days Healing.
Genes with significant changes in expression by ANOVA were compared with genes exhibiting greater than 2-fold changes in expression.

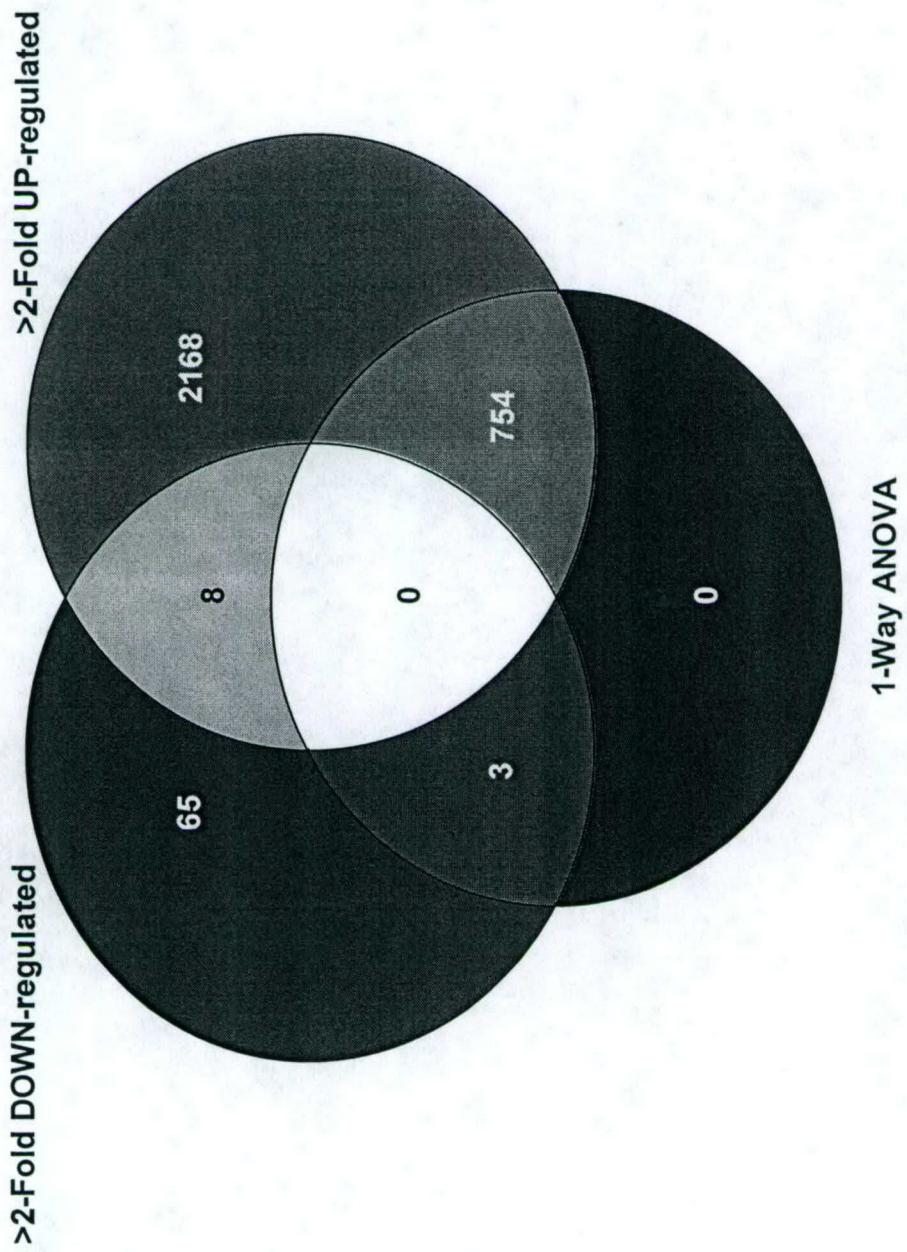
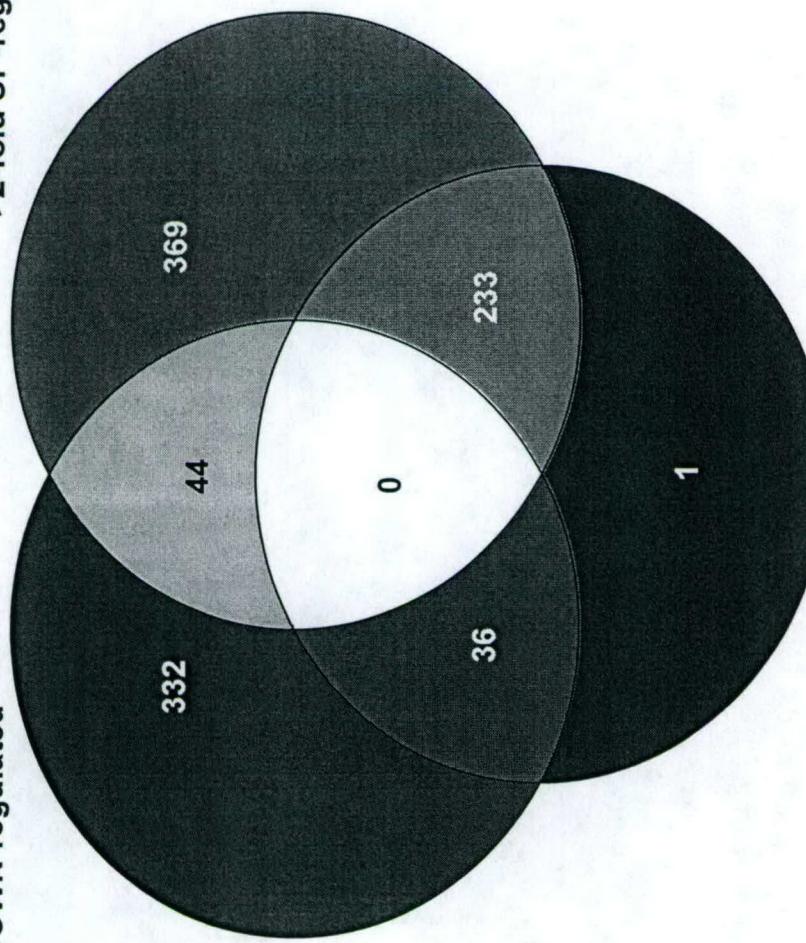


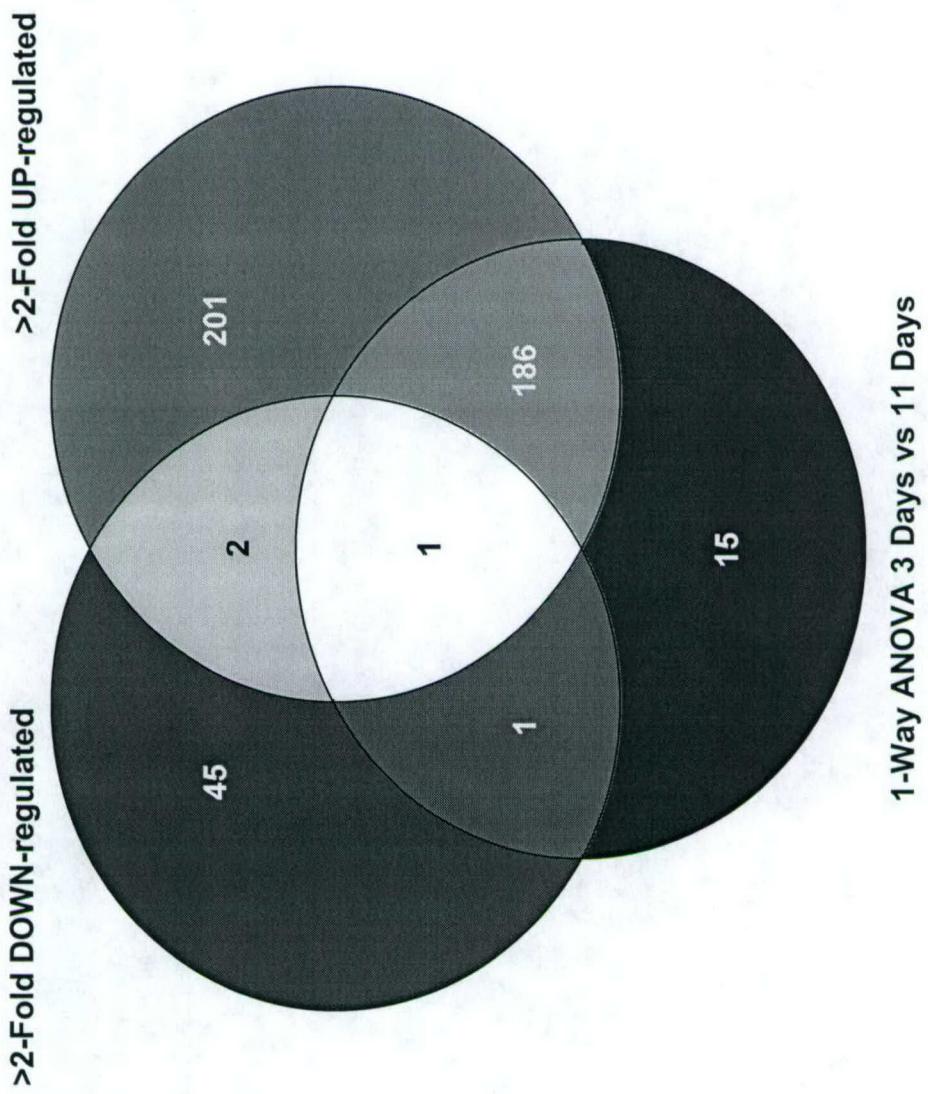
Figure 11: Venn Diagram Illustrating Statistical Analysis of Fracture Gene Expression at 11 Days Healing.
Genes with significant changes in expression by ANOVA were compared with genes exhibiting greater
than 2-fold changes in expression.

>2-fold UP-regulated



1-Way ANOVA

Figure 12: Venn Diagram Illustrating Statistical Analysis of Fracture Gene Expression at 3 Days and 11 Days Healing. Genes with significant changes in expression by ANOVA were compared with genes exhibiting greater than 2-fold changes in expression.



more than 2-fold down-regulated. This data is illustrated in the Venn Diagram (Figure 10) and these genes are listed in Table 1 (Appendix).

2. At 11 days healing, of the 270 genes with expression differences determined to be significant by ANOVA, 233 genes were more than 2-fold up-regulated, and 36 genes were more than 2-fold down-regulated. One was less than 2-fold up- or down-regulated. This data is illustrated in the Venn Diagram (Figure 11) and the genes are listed in Table 2 (Appendix).

3. In a comparison of 3 days versus 11 days healing, of the 203 genes with expression differences determined to be significant by ANOVA, 186 were up-regulated 2-fold, 1 was down-regulated 2-fold, and 1 (defensin NP-2, probably involved in inflammation) was biphasic (up-regulated at one time and down-regulated at the other). Additionally, 15 genes were less than 2-fold up- or down-regulated. This data is illustrated in the Venn Diagram (Figure 12) and the genes are listed in Table 3 (Appendix)

The microarray expression data is now being confirmed by real-time RT-PCR using primer pairs selected for 6 of the known genes and 4 of the Expressed Sequence Tags (ESTs). Primers have been ordered and the fracture RNAs used in the microarray are being reverse transcribed for subsequent PCR confirmation.

4) Conclusions

Microarray analysis of fracture healing identified several genes with significant up-regulation or down-regulation in expression at 3 days healing and 11 days healing. These differences in gene expression will provide insights into the gene pathways that participate in fracture healing, and identify therapeutic gene candidates.

c) Specific Objective 3: To Evaluate the Reproducibility and To Analyze the Data from the Extended Micro-array

1) Objectives

During the second 12 months we will focus on reproducibility of the results and data analyses.

1. Additional animals will be included in the microarray analysis to achieve a sample size of at least 5 individuals (fractured and controls) at 3 days and 11 days healing. This approach will extend the analysis microarray reproducibility of identify individual variations in fracture gene expression.

2. We will confirm the gene expression data in the samples analyzed to date by real-time PCR for genes of interest.

3. We will further analyze the microarray gene expression data to functionally classify the genes with changes in expression, identify gene pathways important in fracture healing and include gene candidate(s) in our fracture therapy.

KEY RESEARCH ACCOMPLISHMENTS

1. We have developed MLV-based and lentiviral-based vector systems for the optimal delivery and expression of therapeutic transgenes to the bone cells of healing fracture tissues.
 - a) The MLV-based vector long-terminal repeat (LTR) provides robust nonspecific constitutive expression of the transgene.
 - b) Two promoters have been identified for transgene expression from the lentiviral-based vector.
 - i) The cytomegalovirus (CMV) promoter provides good nonspecific gene expression.

- ii) Surprisingly, the elongation factor-1a (EF-1a) provided optimal gene-specific expression, superior to the collagen promoters and comparable to the CMV promoter.
- 2. We have optimized surgical procedures to inject the vectors into the interior of the fractured femur through an intramedullary catheter. When compared to simpler injections applied from the exterior aspects of the leg, injection of the BMP-2/4 transgene provided symmetric bone formation within the periosteal layers mediating fracture repair. This system facilitates gene therapy studies by maximizing the effect of the therapy.
- 3. We have performed microarray analysis on the RNA from four individual fracture subjects at two times of healing to examine global gene expression. Several hundred known and unknown genes are being analyzed to identify potential candidate genes for expression from the optimal vector following application to the fracture by the improved delivery technique.

REPORTABLE OUTCOMES

There are no reported outcomes as of this date.

CONCLUSIONS

The development of highly effective gene therapy approaches to musculoskeletal injuries requires the optimization of the components and techniques for the accurate assessment of therapeutic benefits. By identifying which vector and regulatory elements provide the best transgene expression in the bone cells that mediate fracture repair, and by developing vector delivery techniques for optimal therapeutic transgene expression, we have optimized conditions for the accurate evaluation of therapeutic transgene candidates identified by microarray analysis of global gene expression in the normally healing fracture callus. Our previous experience has taught us that if the delivery fails to target expression in suitable cells, even the very robust BMP-2/4 bone forming transgene expressed from an efficient viral vector fails to enhance healing, as measured by bony union of the fracture gap and accelerated return of the bone to prefracture mechanical strength. Other potential therapeutic gene candidates would be expected to fair equally poorly in healing if not targeted accurately. Our studies therefore provide an *in vivo* system for measuring the therapeutic benefits of a particular fracture gene therapy. We have performed the analysis of global fracture gene expression during early healing (3 days) and later healing (11 days). These data reveal several hundred genes with expression increases or decreases of more than 2-fold at each time after fracture. Current examination of these candidate genes from our microarray will provide an opportunity to utilize these genes and the different aspects of our vectors (i.e., constitutive or gene-specific regulation of expression) for therapies specifically designed for maximum therapeutic benefits in bone repair. Combination gene therapy for individual patients with different musculoskeletal injuries can then be developed.

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APPENDICES

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and at 11 Days Fracture Healing

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
207864_Rn	0.049873747	similar to Mouse lysosome-associated protein, transmembrane - 4alpha mRNA, complete cds.
BM144069	0.04980385	unknown function
BI303540	0.049785653	unknown function
NM_012875	0.049695275	Rattus norvegicus Ribosomal protein L39 (Rpl39), mRNA.
NM_013181	0.049589938	Rattus norvegicus Protein kinase, cAMP dependent, regulatory, type 1 (Prkar1a), mRNA.
219279_Rn	0.049411971	Rattus norvegicus preconditioning-inducible gene 1 protein (Pig-1), mRNA.
218896_Rn	0.049380843	Rattus norvegicus general transcription factor IIb (Gtf2b), mRNA.
NM_019301	0.049372596	Rattus norvegicus Complement receptor related protein (Cr1), mRNA.
BE113476	0.049359406	unknown function
291264_Rn	0.049330121	Rattus norvegicus CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2 (Cd36l2), mRNA.
NM_054004	0.049328042	Rattus norvegicus TBP-interacting protein 120A (Tip120A), mRNA.
429753_Rn	0.049283845	unknown function
NM_053912	0.049236423	Rattus norvegicus pleckstrin homology, Sec7 and coiled/coil domains 3 (Psccd3), mRNA.
221824_Rn	0.049219301	similar to Mouse, clone MGC:28735 IMAGE:4460992, mRNA, complete cds.
218445_Rn	0.049157562	Rattus norvegicus 3-hydroxy-3-methylglutaryl CoA lyase (Hmgcl), mRNA.
326761_Rn	0.048991974	similar to Mouse RING-finger protein MURF mRNA, complete cds.
S78556_1	0.048987476	grp75=75 kda glucose regulated protein [rats, Sprague-Dawley, brain, mRNA, 3001 nt].
CB545731	0.048634785	similar to Protein:NP_032846 peptidase 4 [Mus musculus]. score=1.644e-47
NM_139082	0.048593301	Rattus norvegicus BMP and activin membrane-bound inhibitor, homolog (<i>Xenopus laevis</i>) (Bambi), mRNA.
298643_Rn	0.048580058	similar to LIM domains containing protein 1
222433_Rn	0.048548566	similar to myosin binding subunit 85
293026_Rn	0.048481936	R.norvegicus c/ebp gamma mRNA.
217465_Rn	0.048380946	Rattus norvegicus S-Adenosylmethionine decarboxylase 1A (Amd1a), mRNA.
221020_Rn	0.048365627	Rattus norvegicus ribophorin II (Rpn2), mRNA.
221819_Rn	0.048283134	similar to mouse CG14164 gene product, clone MGC:36610 IMAGE:5343888, mRNA, complete cds.
223280_Rn	0.048177796	Rattus norvegicus Nopp140 associated protein (Nap65), mRNA.
NM_022591	0.048166166	Rattus norvegicus telomerase protein component 1 (Tip1), mRNA.
BI292022	0.048002822	unknown function
260322_Rn	0.047851097	similar to Protein:NP_473383 tumor endothelial marker 1 precursor [Mus musculus]. score=1.016e-36
294951_Rn	0.047799231	Rat clone RP31-202M22 strain Brown Norway, complete sequence.
CB548450	0.047677994	similar to Protein:NP_608219 farnesyl diphosphate synthetase [Mus musculus]. score=1.644e-26
208505_Rn	0.047527906	similar to mouse RIKEN cDNA 5830446M03 gene, clone IMAGE:4039369, mRNA, partial cds.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_012925	0.047465077	Rattus norvegicus CD59 antigen (Cd59), mRNA.
BM389346	0.047453156	Rattus norvegicus mRNA for NAD+-specific isocitrate dehydrogenase b-subunit, partial cds.
BQ189915	0.047447532	unknown function
BM391934	0.047122327	Rattus norvegicus pyruvate dehydrogenase kinase 1 (Pdk1), mRNA.
NM_053826	0.047079154	Rattus norvegicus latent transforming growth factor beta binding protein 1 (Ltpbp1), mRNA.
205540_Rn	0.047028693	Rattus norvegicus defensin NP-2 precursor (LOC286995), mRNA.
200837_Rn	0.047027304	Rattus norvegicus peroxiredoxin 2 (Prdx2), mRNA.
NM_017169	0.046996233	similar to Mouse ras-related protein (rab18) mRNA, complete cds.
221374_Rn	0.046804856	unknown function
AI012469	0.046513648	AA817907
223059_Rn	0.046294603	0.046184462
409590_Rn	0.04616796	Rattus norvegicus thioredoxin (Txn), mRNA.
CB545716	0.046080162	Rattus norvegicus estrogen-responsive uterine mRNA, partial sequence.
NM_017050	0.046057137	similar to Translation of nuc:AF394782_1 Homo sapiens rap guanine nucleotide exchange factor mRNA, complete cds; RA-GEF-2, score=7.532e-41
219442_Rn	0.045950022	Rattus norvegicus Superoxide dimutase 1, soluble (Sod1), mRNA.
203663_Rn	0.045824264	similar to Protein:NP_663355 similar to dendritic cell protein; expressed sequence AL022788; expressed sequence AL024247 [Mus musculus], score=5.523e-59
218818_Rn	0.045761383	Rat fos-related antigen DNA, exon 4.
222866_Rn	0.045383821	similar to mouse f-box and WD-40 domain protein 1B, clone IMAGEx:3591748, mRNA, partial cds.
AI101462	0.045383821	unknown function
348275_Rn	0.045340536	Rattus norvegicus B-cell translocation gene 1, anti-proliferative (Btg1), mRNA.
U21719_1	0.045284825	Rattus norvegicus clone D920 intestinal epithelium proliferating cell-associated mRNA sequence.
CB547605	0.045254131	unknown function
327032_Rn	0.045111989	similar to Mouse mRNA for beta-tropomyosin.
NM_053509	0.045072099	Rattus norvegicus zona pellucida glycoprotein 1 (Zp1), mRNA.
AI177116	0.044962004	unknown function
NM_080910	0.044777469	Rattus norvegicus phosphoribosylaminoimidazole succinocarboxamide synthetase (Paics), mRNA.
297831_Rn	0.044302609	similar to Protein:NP_081061 ubiquitin-conjugating enzyme E2C; DNA segment, Chr 2, ERATO Doi 695, expressed [Mus musculus], score=5.976e-81
NM_080902	0.044211492	Rattus norvegicus hypoxia induced gene 1 (Hig1), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_133297	0.044191065	Rattus norvegicus 15-kDa selenoprotein (Sep15-pending), mRNA.
NM_024160	0.044112116	Rattus norvegicus cytochrome b558 alpha-subunit (Cyba), mRNA.
222274_Rn	0.044026874	Rattus norvegicus Vav 1 oncogene (Vav1), mRNA.
BQ191848	0.043679324	unknown function
AF036335.1	0.043637001	Rattus norvegicus NonO/p54nrb homolog mRNA, partial cds.
208278_Rn	0.043630636	similar to Mouse, exostoses (multiple) 2, clone MGC:11478 IMAGE:3965079, mRNA, complete cds.
NM_031811	0.043452894	Rattus norvegicus transaldolase 1 (Taldo1), mRNA.
201982_Rn	0.043326107	similar to Mouse hypoxia induced gene 2 (Hig2) mRNA, complete cds.
291322_Rn	0.043200528	similar to Mouse mRNA for p50b (identical to LSP1 and pp52), complete cds.
297948_Rn	0.042853292	Rat alpha-platelet-derived growth factor receptor mRNA.
348547_Rn	0.042706146	platelet-derived growth factor A-chain (PDGF A-chain) 5 region [rats, macrophage, mRNA Partial, 486 nt].
NM_023972	0.042565763	Rattus norvegicus glutamate transporter EAAC1 interacting protein (Eaac1), mRNA.
NM_133406	0.042514003	Rattus norvegicus 1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, delta) (Agpat4), mRNA.
NM_031785	0.042473435	Rattus norvegicus ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), subunit 1 (Atp6s1), mRNA.
296104_Rn	0.042264073	similar to Mouse SNAG1 (Snag1) mRNA, complete cds.
220651_Rn	0.042217538	Rattus norvegicus Ornithine decarboxylase (Odc1), mRNA.
NM_057132	0.042073032	Rattus norvegicus plynia ras-related homolog A2 (Arha2), mRNA.
297277_Rn	0.04203232	similar to Translation of nuc:AK019081_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:231010B07, full insert sequence; putative. score=6.513e-45
216979_Rn	0.041783945	similar to Protein:NP_080659 RIKEN cDNA 0610011E17 gene [Mus musculus]. score=3.733e-29
205353_Rn	0.041699643	similar to Protein:NP_543027 lymphocyte antigen 6 complex, locus E ligand; DNA segment, Chr 17, Wayne State University 104, expressed [Mus musculus]. score=1.188e-33
203666_Rn	0.041659479	Rattus norvegicus pyruvate dehydrogenase E1 alpha-like (Pdhal), mRNA.
290852_Rn	0.041585475	Rattus norvegicus O6-methylguanine-DNA methyltransferase (Mgmt), mRNA.
293702_Rn	0.04156856	similar to Mouse DNA sequence from clone RP23-292E3 on chromosome 11, complete sequence.
AI574743	0.041542199	similar to Protein:NP_080888 RIKEN cDNA 1810011O01 gene [Mus musculus]. score=7.027e-44
219360_Rn	0.041474699	similar to Mouse mRNA for alpha-2,3-sialyltransferase, complete cds.
NM_012923	0.041409323	Rattus norvegicus Cyclin G1 (Ccng1), mRNA.
219078_Rn	0.041345294	Rattus norvegicus Vascular cell adhesion molecule 1 (Vcam1), mRNA.
217664_Rn	0.041328107	similar to Protein:NP_083902 RIKEN cDNA 2410004H05 [Mus musculus]. score=5.219e-69
AA819021	0.041273904	similar to emb[X97042]MMUBCM4GN M.musculus UBcm4 mRNA, mRNA sequence.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
220518_Rn	0.041231515	Rattus norvegicus succinate-CoA ligase, GDP-forming, alpha subunit (SucIg1), mRNA.
231197_Rn	0.041001706	similar to Mouse DNA sequence from clone RP23-381K21 on chromosome 1, complete sequence.
221045_Rn	0.040981675	Rattus norvegicus Proteasome (prosome, macropain) subunit, beta type, 8 (low molecular mass polypeptide 7) (Psmb8), mRNA.
203152_Rn	0.040893457	similar to Mouse, clone MGC:8206 IMAGE:3590908, mRNA, complete cds.
221154_Rn	0.04067949	similar to Translation of nuc:BC023367_1 Mus musculus, Similar to RIKEN cDNA 2410008H17 gene, clone IMAGE:5027413, mRNA, partial cds. score=7.537e-11
206383_Rn	0.040665523	similar to TFIB related factor hBRF
385112_Rn	0.040601103	Rattus norvegicus ABC50 mRNA, partial cds.
BQ781673	0.040212794	unknown function
CB548411	0.040204551	unknown function
AI598434	0.040198287	unknown function
BF395191	0.04013158	Rat zinc finger binding protein mRNA, complete cds.
337565_Rn	0.040053185	unknown function
AW530773	0.040051594	similar to Protein:NP_033921 calmodulin binding protein 1 [Mus musculus]. score=1.029e-79
293536_Rn	0.039692464	similar to Protein:NP_079758 RIKEN cDNA 2810411G23 gene [Mus musculus]. score=6.854e-59
U17565.1	0.039688277	Rattus norvegicus protein tyrosine phosphatase, non-receptor type 2 (Ptpn2), mRNA.
296230_Rn	0.039633807	Rattus norvegicus intestinal DNA replication protein mRNA, partial cds.
BI274544	0.039467225	similar to Mouse Strain C57BL6/J chromosome 8 BAC, RP23-290L7, Complete Sequence, complete sequence.
219476_Rn	0.039428249	unknown function
291222_Rn	0.039403744	Rattus norvegicus glucocorticoid-induced leucine zipper (Gilz), mRNA.
296009_Rn	0.039397965	similar to data source:SPTR, source key:Q21541, evidence:ISS~putative~related to M142.5 PROTEIN similar to Mouse mRNA for SYT.
223064_Rn	0.039333158	Rattus norvegicus Glutamine synthetase (glutamate-ammonia ligase) (Glul), mRNA.
220754_Rn	0.039282261	similar to Mouse H2-O-like homeo box gene (Hlx) gene, complete cds.
NM_012582	0.039096937	Rattus norvegicus Haptoglobin (Hp), mRNA.
218826_Rn	0.039064177	similar to Mouse, RIKEN cDNA 1110015G04 gene, clone MGC:12050 IMAGE:3707759, mRNA, complete cds.
Y07557.1	0.03895508	R.norvegicus mRNA for olfactory receptor.
NM_012589	0.038950497	Rattus norvegicus Interleukin 6 (interferon, beta 2) (Il6), mRNA.
CA503874	0.038731861	unknown function
221183_Rn	0.038611909	Rattus norvegicus valosin-containing protein (Vcp), mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
AI233262	0.038390083	similar to Protein:NP_077229 RANBP4; EST AA409693 [Mus musculus]. score=1.041e-114
NM_031514	0.038297579	Rattus norvegicus Janus kinase 2 (a protein tyrosine kinase) (Jak2), mRNA.
203092_Rn	0.038208121	similar to Mouse chromosome 11a2 clone rp21-434h10 strain 129S6/SvEvTac, complete sequence.
BQ211607	0.037985594	unknown function
221825_Rn	0.037872296	Rattus norvegicus PKC-delta binding protein (SRBC), mRNA.
220767_Rn	0.037715724	Rattus norvegicus solute carrier family 1, member 3 (Slc1a3), mRNA.
BE120761	0.037671702	Rat mRNA for beta-tubulin T beta15.
NM_021663	0.037625764	Rattus norvegicus NEFA precursor (Nucb2), mRNA.
217773_Rn	0.037563554	similar to Mouse mRNA for hypothetical protein (Tes gene).
CB546432	0.037485823	similar to Translation of nuc:AX23986_1 Sequence 4 from Patent WO0164905; unnamed protein product. score=1.392e-33
298362_Rn	0.037454787	Rattus norvegicus cysteine rich protein 61 (Cyr61), mRNA.
220105_Rn	0.037393903	similar to Protein:NP_079650 RIKEN cDNA 1110008E19 [Mus musculus]. score=6.257e-42
NM_031576	0.037315657	Rattus norvegicus P450 (cytochrome) oxidoreductase (Por), mRNA.
295226_Rn	0.037080998	similar to Mouse, RIKEN cDNA 2810039M17 gene, clone MGC:19258 IMAGE:3982076, mRNA, complete cds.
203115_Rn	0.036943003	similar to mouse hypothetical protein FLJ22329, clone IMAGE:4023108, mRNA.
CB545171	0.036937909	similar to Protein:NP_009148 programmed cell death 10; apoptosis-related protein 15 [Homo sapiens]. score=2.888e-38
295015_Rn	0.036857383	similar to mouse lymphocyte activation-associated protein, clone MGC:30532 IMAGE:5008328, mRNA, complete cds.
221574_Rn	0.036577653	Rattus norvegicus syntenin (Sdcbp), mRNA.
203237_Rn	0.036429976	Rattus norvegicus kinase D-interacting substance of 220 kDa (Kidins220), mRNA.
296100_Rn	0.03641603	unknown function
NM_017055	0.036387031	Rattus norvegicus Transferrin (Tf), mRNA.
435288_Rn	0.036347345	similar to Mouse DLC-1 (Arhgap7) gene, exons 12, 13, and 14 and complete cds.
216733_Rn	0.036287823	similar to Mouse DNA sequence from clone RP23-476D16 on chromosome X, complete sequence.
BU758349	0.036258208	unknown function
218982_Rn	0.03613097	Rattus norvegicus Interleukin 6 receptor (Il6r), mRNA.
201441_Rn	0.035968676	Rat mRNA for dihydrolipoamide succinyltransferase.
298972_Rn	0.035914889	unknown function
297984_Rn	0.035902433	similar to mouse hypothetical protein FLJ10647, clone MGC:36197 IMAGE:3964022, mRNA, complete cds.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
AW915841	0.035781569	unknown function
217477_Rn	0.03574078	Rattus norvegicus prostaglandin F2 receptor negative regulator (Ptgfrn), mRNA.
BQ209715	0.035576904	unknown function
220427_Rn	0.035481495	similar to Mouse, clone IMAGE:3991175, mRNA, partial cds.
347777_Rn	0.035335977	similar to Mouse, RIKEN cDNA 1500031N16 gene, clone MGC:11632 IMAGE:3582724, mRNA, complete cds.
199999_Rn	0.035307226	Rat mRNA for ribosomal protein L39.
NM_057191	0.035220344	Rattus norvegicus sarcomeric muscle protein (Sarcosin), mRNA.
AA925053	0.035157646	similar to gi 1478204 emb X97490 MMPNGPROT M.musculus mRNA for PNG protein, mRNA sequence.
222782_Rn	0.035128971	Rattus norvegicus heterogeneous nuclear ribonucleoprotein K (Hnprk), mRNA.
BF283659	0.0350206	unknown function
AI234016	0.035019155	similar to GCGPROT:MCM4_MOUSE DNA REPLICATION LICENSING FACTOR MCM4 (CDC21 HOMOLOG) (P1-CDC21). score=4.351e-44
NM_130416	0.034965935	Rattus norvegicus annexin A7 (Anxa7), mRNA.
218116_Rn	0.034962389	Rattus norvegicus vacuolar proton-ATPase subunit M9.2 (Atp6k), mRNA.
322787_Rn	0.034849902	similar to Mouse SF3b1 mRNA for pre-mRNA splicing factor SF3b 155 kDa subunit, complete cds.
199973_Rn	0.034715539	Rattus norvegicus protein tyrosine phosphatase, non-receptor type 6 (Ptph6), mRNA.
AI233916	0.034621057	unknown function
X94509	0.034613492	unknown function
220547_Rn	0.034539517	Rattus norvegicus Fumarate hydratase (Fh), mRNA.
223321_Rn	0.034513757	Rattus norvegicus NF-E2-related factor 2 (Nfe2l2), mRNA.
384573_Rn	0.034377536	Rattus norvegicus maternal G10 transcript (G10), mRNA.
294517_Rn	0.034339947	similar to mouse RIKEN cDNA 3830408P04 gene, clone IMAGE:3601026, mRNA, partial cds.
AA819350	0.034177255	similar to Translation of nuc:BC022119_1 Mus musculus, clone MGC:37737 IMAGE:5067385, mRNA, complete cds. score=9.304e-55
295272_Rn	0.034132278	unknown function
293680_Rn	0.033859253	Rattus norvegicus guanine nucleotide binding protein, alpha 15 (Gna15), mRNA.
297715_Rn	0.033820132	Rat mRNA for p34 protein, complete cds.
NM_031660	0.033651684	Rattus norvegicus cyclic AMP phosphoprotein, 19 kDa (Arpp19), mRNA.
205764_Rn	0.03336253	similar to Mouse cytosolic beta-N-acetylglucosaminidase (Mgea5) mRNA, complete cds.
BE117350	0.033319145	unknown function
NM_012848	0.033229416	Rattus norvegicus Ferritin subunit H (Fth1), mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
208158_Rn	0.033195242	Rattus norvegicus solute carrier family 7 (cationic amino acid transporter, y+system), member 7 (Slc7a7), mRNA.
BQ192866	0.033142974	unknown function
234932_Rn	0.033136104	similar to Translation of nuc:U22437_1 Mus cookii steroid receptor mRNA, partial cds. score=6.427e-41
295783_Rn	0.033043832	Rat UDP-glucose glycoprotein:glucosyltransferase precursor (Uggt) mRNA, complete cds.
217792_Rn	0.032783322	unknown function
220185_Rn	0.03273315	similar to Protein:NP_598865 expressed sequence A1256693 [Mus musculus]. score=1.873e-41
NM_031056	0.032711326	Rattus norvegicus matrix metalloproteinase 14, membrane-inserted (Mmp14), mRNA.
222663_Rn	0.032456113	similar to Mouse, clone MGC:38921 IMAGE:5362560, mRNA, complete cds.
295983_Rn	0.032377602	similar to Mouse QKI protein (qkl) gene, alternative splice product, exon 9 and complete cds.
220595_Rn	0.032352294	similar to Protein:NP_064681 tumor-suppressing subchromosomal transferable fragment 4; ESTM671070 (Roswell Park); EST AA241958 [Mus musculus]. score=9.712e-44
219223_Rn	0.032306843	Rattus norvegicus nuclear distribution gene C homolog (Aspergillus) (Nudc), mRNA.
A1009167	0.032302824	unknown function
222083_Rn	0.032198023	similar to Mouse chromosome 10 clone rp21-668b24 strain 129S6/SvEvTac, complete sequence.
L25387_1	0.032122111	Rat phosphofructokinase C (PFK-C) mRNA, complete cds.
200279_Rn	0.032113202	Rattus norvegicus acidic ribosomal protein P0 (Arbp), mRNA.
222666_Rn	0.032093234	Rattus norvegicus liver cytochrome c oxidase subunit VIII (COX-VIII) mRNA, 3 end of cds.
NM_031974	0.032055465	Rattus norvegicus clathrin, light polypeptide (Lca) (Cita), mRNA.
215948_Rn	0.031874071	similar to Similar to HTPAP protein
208191_Rn	0.031819911	Rattus norvegicus guanine nucleotide binding protein gamma 10 subunit mRNA, partial cds.
BI275243	0.031674894	similar to Protein:NP_598819 expressed sequence AA409897 [Mus musculus]. score=1.544e-84
AA818093	0.031665918	unknown function
217333_Rn	0.031433569	similar to Mouse, clone MGC:6737 IMAGE:3590767, mRNA, complete cds.
220486_Rn	0.031406025	unknown function
222092_Rn	0.031311556	Rattus norvegicus ferritin light chain 1 (Ftl1), mRNA.
413727_Rn	0.0310449	R..rattus mRNA for ribosomal protein L11.
207650_Rn	0.030874539	similar to breast cancer associated protein BRAP1
NM_138892	0.030791995	Rattus norvegicus RS21-C6 protein (RS21-C6), mRNA.
200127_Rn	0.030781858	Rattus norvegicus Secreted acidic cystein-rich glycoprotein (osteonectin) (Sparc), mRNA.
199918_Rn	0.030454969	Rattus norvegicus ribosomal protein L29 (Rpl29), mRNA. GCGEST:BQ205444
296074_Rn	0.030438962	similar to Translation of nuc:AF414190_1 Mus musculus embryonic seven-span transmembrane protein-like protein (J207) mRNA, complete cds. score=2.121e-39

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_139105	0.030392428	Rattus norvegicus ribonuclease/angiogenin inhibitor (Rnh1), mRNA.
BE120644	0.030370366	unknown function
CA334630	0.030206327	similar to Protein:NP_083000 RIKEN cDNA 4632403N06 [Mus musculus]. score=1.182e-104
199497_Rn	0.030065838	Rattus norvegicus stearoyl-Coenzyme A desaturase 2 (Scd2), mRNA.
NM_021989	0.02998075	Rattus norvegicus tissue inhibitor of metalloproteinase 2 (Timp2), mRNA.
204455_Rn	0.029967903	similar to Protein:NP_079660 RIKEN cDNA 1110003P16 gene [Mus musculus]. score=2.735e-64
204926_Rn	0.029867939	similar to Mouse groucho-related protein (Grg2) mRNA, complete cds.
NM_022503	0.029781852	Rattus norvegicus cytochrome c oxidase, subunit 7a 3 (Cox7a3), mRNA.
NM_013043	0.029739688	Rattus norvegicus Transforming growth factor beta stimulated clone 22 (Tgfb14), mRNA.
206040_Rn	0.029701379	similar to Mouse thymidine kinase gene, complete cds.
218405_Rn	0.029699099	unknown function
BM387285	0.029677601	unknown function
205354_Rn	0.029630758	Rattus norvegicus peroxiredoxin 4 (Prdx4), mRNA.
199584_Rn	0.02963003	similar to Mouse, tumor protein D52, clone MGC:8210 IMAGE:3590969, mRNA, complete cds.
BQ211631	0.029577151	unknown function
217299_Rn	0.029473166	Rat mRNA for neuron glucose transporter.
CA509361	0.029323917	similar to Protein:NP_000875 IMP (inosine monophosphate) dehydrogenase 2; IMP (inosine 5'-phosphate) dehydrogenase-2 [Homo sapiens]. score=5.184e-83
AA875619	0.029322804	unknown function
298704_Rn	0.029141386	similar to TSP2=thrombospondin 2 [Mouse, Genomic, 2030 nt, segment 2 of 2].
291340_Rn	0.029118517	Rat jun dimerization protein 2 (Jdp-2) mRNA, complete cds.
203647_Rn	0.028909883	similar to Protein:NP_075709 RIKEN cDNA 2010106G01 gene [Mus musculus]. score=1.537e-26
AA899819	0.0288838137	similar to gij57070 emb X05300 RNRIBI Rat mRNA for ribophorin I, mRNA sequence.
291940_Rn	0.028778797	similar to Mouse DNA sequence from clone RP23-317H19 on chromosome X, complete sequence.
218806_Rn	0.028733811	Rattus norvegicus regulator of G-protein signalling protein 2 (Rgs2), mRNA.
BQ781402	0.028721361	unknown function
NM_031789	0.028610444	Rattus norvegicus NF-E2-related factor 2 (Nfe2l2), mRNA.
297796_Rn	0.028496929	similar to Mouse DNA sequence from clone RP23-74L9 on chromosome 11, complete sequence.
292843_Rn	0.028434412	similar to Mouse, a disintegrin and metalloprotease domain 8, clone MGC:36123 IMAGE:5324943, mRNA, complete cds.
AA925418	0.028349207	unknown function
BQ205032	0.028304612	unknown function
219697_Rn	0.028188134	similar to Mouse chromosome 5 clone rp23-268d19 strain C57BL/6J, complete sequence.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
BU759576	0.028088111	unknown function
BQ210493	0.027920074	similar to Protein:NP_080048 dystrobrevin binding protein 1 [Mus musculus]. score=3.223e-91
206212_Rn	0.027665648	Rattus norvegicus actinin alpha 2 associated LIM protein (Pdlim3), mRNA.
CB545220	0.027577351	similar to Protein:NP_082359 RIKEN cDNA 2600017H24 [Mus musculus]. score=1.228e-26
NM_138888	0.027524435	Rattus norvegicus transcription factor INI (Loc192246), mRNA.
217006_Rn	0.027435695	Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 1 (Psma1), mRNA.
295692_Rn	0.02738032	similar to Mouse mRNA for src-like adaptor protein.
298215_Rn	0.027317576	similar to Mouse tumor endothelial marker 8 precursor (Tem8) mRNA, complete cds.
NM_022402	0.027048908	Rattus norvegicus acidic ribosomal protein P0 (Arbp), mRNA.
NM_031151	0.026994764	Rattus norvegicus malate dehydrogenase mitochondrial (Mor1), mRNA.
CA340775	0.026951519	unknown function
217547_Rn	0.026906478	Rattus norvegicus Histone H1-0 (H1f0), mRNA.
CA509996	0.026906349	unknown function
NM_012895	0.026837451	Rattus norvegicus Adenosin kinase (Adk), mRNA.
204841_Rn	0.026811784	similar to Mouse, RIKEN cDNA 2510048O06 gene, clone MGC:35726 IMAGE:5375452, mRNA, complete cds.
206085_Rn	0.026635624	similar to Mouse MRPL33 mRNA for mitochondrial ribosomal protein L33 (L33mt), complete cds.
206530_Rn	0.026587343	Rattus norvegicus UNC-119 homolog (C. elegans) (Unc119), mRNA.
348004_Rn	0.026477174	similar to Mouse heterogenous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) mRNA, complete cds.
NM_017325	0.026474744	Rattus norvegicus Runt related transcription factor 1 (Runx1), mRNA.
219250_Rn	0.026377182	similar to Translation of nuc:X67029_1 B.taurus mRNA for guanylate kinase. score=1.144e-78
BQ203725	0.026305021	R.norvegicus mRNA for hypoxanthine-guanine phosphoribosyltransferase.
328103_Rn	0.026183539	Rattus norvegicus stem cell derived neuronal survival protein precursor (Sdnsf), mRNA.
BU760017	0.025988392	similar to GCGPROT:Q9UNV9 PUTATIVE RNA HELICASE. score=2.006e-97
219111_Rn	0.025722012	Rattus norvegicus proteasome (prosome, macropain) subunit, beta type, 2 (Psmb2), mRNA.
NM_053365	0.025711621	Rattus norvegicus fatty acid binding protein 4 (Fabp4), mRNA.
BQ207009	0.025690964	unknown function
220172_Rn	0.025631959	Rattus norvegicus -ral simian leukemia viral oncogene homolog A (ras related) (RalA), mRNA.
BQ781097	0.025596293	unknown function
AI502470	0.025491033	similar to Translation of nuc:BC004667_1 Mus musculus, clone IMAGE:3498604, mRNA, partial cds.
CA506853	0.025484672	score=1.538e-28
286501_Rn	0.025459859	similar to Mouse DNA sequence from clone RP23-151M22 on chromosome 11, complete sequence.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
218104_Rn	0.025363599	Rattus norvegicus copper chaperone for superoxide dismutase (Ccs), mRNA.
216246_Rn	0.025326332	Rattus norvegicus thioredoxin-like 2 (Txnl2), mRNA.
384113_Rn	0.025306869	Rattus norvegicus complement component factor h (Cfh), mRNA.
NM_053556	0.025139859	Rattus norvegicus maternal G10 transcript (G10), mRNA.
A1171953	0.025085186	Rattus norvegicus putative ionotropic glutamate receptor GLURR-F11694B (Glurr) mRNA, partial cds.
NM_053969	0.025062582	Rattus norvegicus G protein pathway suppressor 1 (Gps1), mRNA.
NM_012963	0.025031751	Rattus norvegicus high mobility group box 1 (Hmgb1), mRNA.
NM_031557	0.024876828	Rattus norvegicus Prostaglandin I2 (prostacyclin) synthase (Ptgis), mRNA.
275389_Rn	0.02485342	similar to mouse electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II), clone MGC:6481 IMAGE:2646522, mRNA, complete cds.
200736_Rn	0.024851507	Rattus norvegicus myosin heavy chain, polypeptide 6, cardiac muscle, alpha (Myh6), mRNA.
288722_Rn	0.024848495	similar to Mouse mRNA for mPACPL1, complete cds.
NM_024351	0.0248231	Rattus norvegicus Heat shock cognate protein 70 (Hsc70), mRNA.
201624_Rn	0.024557296	similar to Translation of nuc:BC025529_1 Mus musculus, Similar to splicing factor, arginine/serine-rich 7 (35kD), clone MGC:38287 IMAGE:5342587, mRNA, complete cds. score=7.527e-07
203101_Rn	0.024513916	similar to Mouse, cytochrome c oxidase subunit VIIb, clone MGC:35766 IMAGE:4987472, mRNA, complete cds.
222207_Rn	0.024236938	similar to Translation of nuc:BC008511_1 Mus musculus, Similar to eukaryotic translation initiation factor 3, subunit 4 (delta, 44kD), clone MGC:5726 IMAGE:3592537, mRNA, complete cds. score=2.539e-34
L19927_1	0.024071364	Rattus norvegicus (clone gamma-3) ATP synthase gamma-subunit (ATP5c) mRNA, 3 end cds.
A1100773	0.024017788	unknown function
NM_053319	0.023981514	Rattus norvegicus dynein, cytoplasmic, light chain 1 (Pin), mRNA.
221408_Rn	0.023731648	unknown function
NM_019259	0.02360757	Rattus norvegicus complement component 1, q subcomponent binding protein (C1qbp), mRNA.
294991_Rn	0.023583219	similar to Mouse, eukaryotic translation initiation factor 3, subunit 6 (48kD), clone MGC:36795 IMAGE:3988386, mRNA, complete cds.
348489_Rn	0.023450656	similar to Protein:NP_079802 RIKEN cDNA 2310020H20 [Mus musculus]. score=2.503e-66
290197_Rn	0.023321534	similar to evidence:NAS~hypothetical protein~putative
NM_138548	0.023291982	Rattus norvegicus expressed in non-metastatic cells 1 (Nme1), mRNA.
NM_017281	0.02327355	Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 4 (Psma4), mRNA.
290927_Rn	0.023169282	unknown function
284666_Rn	0.02295409	similar to Mouse mRNA for p40phox, complete cds.
221399_Rn	0.022943249	Rat mRNA for proteasome subunit RCX, complete cds.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
203486_Rn	0.022813287	similar to mouse dysterlin, clone IMAGE:5324940, mRNA, partial cds.
218928_Rn	0.022687634	similar to Mouse enhancer of rudimentary homolog mRNA, complete cds.
NM_031756	0.022629894	Rattus norvegicus gamma-glutamyl carboxylase (Ggcx), mRNA.
348360_Rn	0.022597414	Rattus norvegicus Nogo-A (Rtn4), mRNA.
NM_017264	0.022513294	Rattus norvegicus protease (prosome, macropain) 28 subunit, alpha (Psme1), mRNA.
216942_Rn	0.022373898	similar to mouse hypothetical protein FLJ14753, clone MGC:25756 IMAGE:3993056, mRNA, complete cds.
CB546087	0.022319121	similar to Translation of nuc:AF048695_1 <i>Mus musculus</i> type I alpha phosphatidylinositol-4-phosphate 5-kinase variant (Pip5ka) mRNA, complete cds. score=2.799e-60
NM_019201	0.022260752	Rattus norvegicus C-terminal binding protein 1 (Ctbp1), mRNA.
AA858962	0.022174933	Rat retinol-binding protein (RBP) mRNA, partial cds.
206962_Rn	0.02194767	similar to Mouse adenylosuccinate synthetase mRNA, complete cds.
219418_Rn	0.021945732	similar to Mouse 7 BAC RP23-266F22 (Roswell Park Cancer Institute Mouse BAC Library) complete sequence.
199762_Rn	0.021771221	similar to Mouse, nascent-polypeptide-associated complex alpha polypeptide, clone MGC:35995 IMAGE:3992262, mRNA, complete cds.
221202_Rn	0.021744546	Rattus norvegicus solute carrier family 2 (facilitated glucose transporter) member 1 (Slc2a1), mRNA.
BM387852	0.021625873	unknown function
222626_Rn	0.021601117	similar to Translation of nuc:AK017221_1 <i>Mus musculus</i> 10 days neonate intestine cDNA, RIKEN full-length enriched library, clone:5133400M17, full insert sequence; putative. score=1.652e-69
203454_Rn	0.0215208	similar to Protein:NP_035792 TYRO protein tyrosine kinase binding protein; killer cell activating receptor associated protein [Mus musculus]. score=6.358e-41
218564_Rn	0.021507687	similar to Mouse mRNA for mMCM2, complete cds.
296435_Rn	0.021450941	Rattus norvegicus glycogenin (Gyg), mRNA.
NM_130413	0.021431162	Rattus norvegicus src family associated phosphoprotein 2 (Scap2), mRNA.
296477_Rn	0.021315583	similar to Mouse, RIKEN cDNA 2610034B18 gene, clone MGC:388839 IMAGE:5360750, mRNA, complete cds.
216802_Rn	0.021231781	Rattus norvegicus Hermansky-Pudlak syndrome (Hps), mRNA.
AA955326	0.021231683	similar to gil57719[emb]X51537 RRRPS20 Rat mRNA for ribosomal protein S20, mRNA sequence.
AB016165_1	0.021155007	Rattus norvegicus Tsc1 mRNA, 5-noncoding region.
BM387331	0.021126558	similar to Protein:NP_080594 RIKEN cDNA 2310003F16 gene [Mus musculus]. score=4.646e-36
322818_Rn	0.021072777	similar to Mouse, clone IMAGE:3489640, mRNA.
298068_Rn	0.020784248	Rattus norvegicus CCAAT/enhancerbinding, protein (C/EBP) delta (Cebpd), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
201585_Rn AW434065	0.020607092 0.020514706	similar to Mouse, clone IMAGE:4921359, mRNA. unknown function
BE128197	0.020276357	similar to Protein:NP_035946 Ras-GTPase-activating protein (GAP<120>) SH3-domain binding protein 2; ras-GTPase-activating protein (GAP<120>) SH3-domain-binding protein 2 [Mus musculus]. score=6.753e-50
206462_Rn 223254_Rn 218113_Rn 291836_Rn	0.02024707 0.020198983 0.020192936 0.019720075	Rat chromosome 6 clone RP31-263K14 strain Brown Norway, complete sequence. similar to Mouse mRNA for huntingtin interacting protein-2, complete cds. similar to Protein:NP_033486 ubiquitin-like 1 [Mus musculus]. score=4.828e-52 similar to Protein:NP_079979 RIKEN cDNA 3930402F23 gene [Mus musculus]. score=5.076e-35
NM_057119	0.019584901	Rattus norvegicus splicing factor, arginine/serine-rich (transformer 2 Drosophila homolog) 10 (Sfrs10), mRNA.
199737_Rn 223058_Rn 203128_Rn NA	0.019555472 0.019544902 0.019480205 0.01938376	similar to mouse cyclin B2, clone MGC:11566 IMAGE:3156950, mRNA, complete cds. similar to Protein:NP_064363 heat shock protein 030 [Mus musculus]. score=1.136e-36 Rattus norvegicus fertility protein SP22 (SP22), mRNA.
429760_Rn NM_022634 NM_021765 218111_Rn 218793_Rn 297791_Rn BM383514 218610_Rn 297800_Rn NM_031119 NM_017280 CB545156 AI410339 205991_Rn NM_019249 363623_Rn	0.019373379 0.019337953 0.019321867 0.019307362 0.019282319 0.019264057 0.01897211 0.018957181 0.018890147 0.018816911 0.018797168 0.018729412 0.018691665 0.018682842 0.018550463 0.018518189	antiuitinin=26g turgor protein homolog [rats, intestinal mucosa, mRNA Partial, 879 nt]. Rattus norvegicus leucocyte specific transcript 1 (Lst1), mRNA. Rattus norvegicus beta prime COP (COPb), mRNA. similar to Mouse, clone MGC:8028 IMAGE:3586619, mRNA, complete cds. Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 4 (Psma4), mRNA. similar to cyclin-dependent kinase inhibitor 3 unknown function similar to Mouse, clone IMAGE:5011849, mRNA. Rattus norvegicus outer mitochondrial membrane receptor rTOM20 (LOC2666601), mRNA. Rattus norvegicus Sjogren syndrome antigen B (Ssb), mRNA. Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 3 (Psma3), mRNA. similar to Translation of nuc:AF426024_1 Mus musculus ElA (Serpib1) mRNA, complete cds; ov-serpin; MNEI-like protein A. score=1.433e-70 unknown function similar to Mouse, RIKEN cDNA 1500002O20 gene, clone MGC:37579 IMAGE:4988361, mRNA, complete cds. Rattus norvegicus protein tyrosine phosphatase, receptor type, F (Ptprf), mRNA. Rattus norvegicus ATPase, Na+K+ transporting, beta polypeptide 3 (Atp1b3), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
295314_Rn	0.018509705	similar to Translation of nuc:BC004064_1 Mus musculus, clone lIMAGE:3590584, mRNA, partial cds. score=5.817e-32
201293_Rn	0.018332298	similar to SPF31
BM388494	0.018293157	unknown function
NM_019331	0.017971422	Rattus norvegicus Proprotein convertase subtilisin/kexin type 3 (paired basic amino acid cleaving enzyme, furin, membrane associated receptor protein) (Pcsk3), mRNA.
202269_Rn	0.01787831	unknown function
NM_031112	0.017829384	Rattus norvegicus ribosomal protein S24 (Rps24), mRNA.
216165_Rn	0.017782638	similar to Mouse mRNA for ASM-like phosphodiesterase 3a.
NM_017277	0.017690498	Rattus norvegicus adaptor protein complex AP-1, beta 1 subunit (Ap1b1), mRNA.
NM_013065	0.017533846	Rattus norvegicus Protein phosphatase 1, catalytic subunit, beta isoform (Ppp1cb), mRNA.
217876_Rn	0.017533391	similar to Mouse (SRP9) signal recognition particle subunit mRNA, 689bp.
NM_012985	0.017511332	Rattus norvegicus NADH ubiquinone oxidoreductase subunit B13 (Ndufa5), mRNA.
BQ194792	0.017436153	similar to Protein:NP_079663 RIKEN cDNA 1110021D01 gene [Mus musculus]. score=8.434e-37
207121_Rn	0.017350838	similar to Protein:NP_081115 RIKEN cDNA 1110055E19 [Mus musculus]. score=2.285e-08
217022_Rn	0.017269639	similar to Mouse mRNA for Rab7 protein.
206358_Rn	0.017225005	similar to Sushi domain (SCR repeat) containing protein~data source:Pfam, source key:PF00084, evidence:ISS~putative fibroblast growth factor receptor 1 beta-isoform [Rattus norvegicus=Norway rat, Sprague-Dawley, kidneys, mRNA, 2520 nt].
384793_Rn	0.017084658	similar to Mouse, clone IMAGE:4221113, mRNA.
218337_Rn	0.01705073	Rattus norvegicus Sterol carrier protein 2, liver (Scp2), mRNA.
217960_Rn	0.016989747	Rat mRNA for T-plastin.
204888_Rn	0.016872883	Rattus norvegicus Superoxide dimutase 1, soluble (Sod1), mRNA.
221804_Rn	0.016826237	similar to Mouse IQ motif containing GTPase activating protein 1 (Iqgap1) mRNA, complete cds.
221829_Rn	0.016441098	Rattus norvegicus heat shock protein 90 beta mRNA, partial sequence.
329636_Rn	0.016415667	similar to Translation of nuc:X95906_1 B.taurus mRNA for CPSF (cleavage and polyadenylation specificity factor) 73 kDa subunit; 73 kDa subunit. score=4.234e-81
288315_Rn	0.016378518	Rattus norvegicus sorting nexin 1 (Snx1), mRNA.
217292_Rn	0.016247993	Rattus norvegicus Cystatin beta (Cstb), mRNA.
201347_Rn	0.016241167	Rattus norvegicus Carboxypeptidase E (Cpe), mRNA.
223283_Rn	0.016119724	Rattus norvegicus connective tissue growth factor (Ctgf), mRNA.
298840_Rn	0.016032621	unknown function
CB546220	0.01593386	

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
207079_Rn	0.015914064	similar to Mouse clone mgs1-182e11 strain 129/SvJ, complete sequence.
217237_Rn	0.015871243	Rattus norvegicus vitamin A-deficient testicular protein 2-like mRNA, partial sequence.
221731_Rn	0.015868948	Rattus norvegicus retinoblastoma binding protein 7 (Rbbp7), mRNA.
204302_Rn	0.015796911	similar to Translation of nuc:AK010720_1 Mus musculus ES cells cDNA, RIKEN full-length enriched library, clone:2410073P13, full insert sequence; putative. score=0
217587_Rn	0.015729616	Rattus norvegicus D123 gene product (D123), mRNA.
297939_Rn	0.015724065	similar to Mouse DNA sequence from clone RP23-278I3 on chromosome 4, complete sequence.
NM_017173	0.015424301	Rattus norvegicus serine (or cysteine) proteinase inhibitor, clade H, member 1 (Serpinh1), mRNA.
313371_Rn	0.015319657	unknown function
296312_Rn	0.015311252	Rattus norvegicus Apolipoprotein B editing protein (Apobec1), mRNA.
BE097398	0.015189701	unknown function
CA508165	0.01518025	unknown function
200856_Rn	0.015137468	similar to Translation of nuc:AF375046_1 Mus musculus ATP-dependent chromatin remodeling protein SNF2H mRNA, complete cds; similar to human SNF2H. score=0
216407_Rn	0.015120898	Rattus norvegicus calpastatin I heavy chain (Anxa2), mRNA.
220314_Rn	0.015075422	similar to Protein:NP_081403 RIKEN cDNA 2310016C16 [Mus musculus]. score=3.545e-36
218557_Rn	0.015061062	similar to Mouse (Mus musculus domesticus) mitochondrial carrier homolog 2 mRNA, complete cds; nuclear gene for mitochondrial product.
200179_Rn	0.015034271	UI-R-A0-be-c-08-0-UI.s1 UI-R-A0 Rattus norvegicus cDNA clone UI-R-A0-be-c-08-0-UI 3 similar to gbl12459 RATLYSOZYM Rat lysozyme gene exons 1-4, complete cds, mRNA sequence.
BE104361	0.014990979	Rat CIIIBBP (homologous to human SSRP-1 and mouse T160 genes) mRNA, 3 end.
295616_Rn	0.014933537	similar to Mouse BCL3 (Bcl3) mRNA, complete cds.
223327_Rn	0.014910958	similar to Unknown (protein for IMAGE:3956746)
NM_012839	0.014818899	Rattus norvegicus cytochrome c, somatic (Cycs), mRNA.
NM_022516	0.014816732	Rattus norvegicus polypyrimidine tract binding protein (Ptbp), mRNA.
202313_Rn	0.014805995	Rattus norvegicus Cell division cycle control protein 2 (Cdc2a), mRNA.
NM_012756	0.014519329	Rattus norvegicus insulin-like growth factor 2 receptor (Igf2r), mRNA.
219126_Rn	0.014420798	Rattus norvegicus ribosomal protein S13 (RPS13) mRNA, 3 end.
BE126741	0.014379768	Rattus norvegicus clone p9.2 tenascin mRNA, partial cds.
221559_Rn	0.01435988	Rattus norvegicus dolichol-phosphate (beta-D) mannosyltransferase 2 (Dpm2), mRNA.
NM_133594	0.014093826	Rattus norvegicus SMT3 suppressor of mif two 3 homolog 2 (yeast) (Smt3h2), mRNA.
NM_134346	0.014078799	Rattus norvegicus RAP1B, member of RAS oncogene family (Rap1b), mRNA.
215862_Rn	0.013951178	similar to Mouse DNA sequence from clone RP23-480B19 on chromosome 13, complete sequence.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
199952_Rn	0.01387258	Rattus norvegicus ribosomal protein S17 (Rps17), mRNA.
222334_Rn	0.013804176	Rat Rieske iron-sulfur protein mRNA, complete cds.
221318_Rn	0.013758427	unknown function
BQ208511	0.013709766	unknown function
220377_Rn	0.013639749	similar to Mouse, FK506-binding protein 3 (25kD), clone MGC:6631 IMAGE:3492535, mRNA, complete cds.
288484_Rn	0.013561995	similar to Translation of nuc:AB037738_1 Homo sapiens mRNA for KIAA1317 protein, partial cds; Start codon is not identified.. score=8.928e-45
NM_031706	0.013537243	Rattus norvegicus ribosomal protein S8 (Rps8), mRNA.
298340_Rn	0.013513059	unknown function
BQ782951	0.013498257	similar to Translation of nuc:U58888_1 Mus musculus SH3-containing protein SH3P2 mRNA, partial cds; contains SH3 domain and ankyrin repeats. score=2.1e-104
A1712699	0.013440739	unknown function
208234_Rn	0.013435548	Rattus norvegicus coronin, actin binding protein 1A (Coro1a), mRNA.
206189_Rn	0.013342253	similar to Translation of nuc:AY013699_1 Mus musculus FKSG24 (Fksg24) mRNA, complete cds. score=1.021e-07
215769_Rn	0.013296633	Rattus norvegicus global ischemia induced protein Gllg15B (Gllg15b), mRNA.
222535_Rn	0.0132329693	Rattus norvegicus peroxiredoxin 2 (Prdx2), mRNA.
297849_Rn	0.013170786	similar to Mouse DNA sequence from clone RP23-353J21 on chromosome 11, complete sequence.
220117_Rn	0.013140271	similar to Mouse lymphotoxin-beta receptor gene, putative promoter and exons 1,2,3,4,5 and 6, partial cds.
296375_Rn	0.013048524	similar to Mouse NUF2R mRNA, complete cds.
BE110980	0.012934381	unknown function
219156_Rn	0.012773536	unknown function
202441_Rn	0.012727495	similar to retinoic acid receptor responder (tazarotene induced) 1
NM_130405	0.012690805	Rattus norvegicus src associated in mitosis, 68 kDa (Sam68), mRNA.
NM_017097	0.012577901	Rattus norvegicus Cathepsin C (dipeptidyl peptidase I) (Ctsc), mRNA.
NM_022385	0.012563775	Rattus norvegicus ADP-ribosylation factor-like 1 (Arl1), mRNA.
NM_133416	0.012504606	Rattus norvegicus BCL2-related protein A1 (Bcl2a1), mRNA.
200838_Rn	0.012504231	Rattus norvegicus Actin, gamma 2, smooth muscle, enteric (Actg2), mRNA.
260281_Rn	0.012456801	Rat collagenase (UMRCCase) mRNA, 3 end.
CA509438	0.012450221	unknown function
297861_Rn	0.012364873	similar to Sm D2

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_0222592	0.0122266134	Rattus norvegicus transketolase (Tkt), mRNA.
298172_Rn	0.012216327	similar to mouse growth factor receptor bound protein 10, clone MGC:28740 IMAGE:4481345, mRNA, complete cds.
CB548029	0.012177883	similar to Translation of nuc:U55935_1 Canis familiaris tight junction associated protein ZO-1 MDCK (ZO1-MDCK) mRNA, complete cds; Zonula occludens 1, membrane associated guanylate kinase, MDCK; Tight Junction associated protein (MAGUK). score=1.349e-78
295478_Rn	0.012121735	similar to Mouse, RIKEN cDNA 4833419j07 gene, clone MGC:31193 IMAGE:4194996, mRNA, complete cds.
221375_Rn	0.01211142	similar to Mouse, clone MGC:18674 IMAGE:4207201, mRNA, complete cds.
201836_Rn	0.012056347	Rat mRNA for ribosomal protein L7a.
199390_Rn	0.012024097	Rattus norvegicus high mobility group-17 mRNA, 3 untranslated region, partial sequence.
219944_Rn	0.012006099	Rat pre-pro-insulin-like growth factor mRNA, class A, 5 end.
293161_Rn	0.011999199	Rattus norvegicus rabphilin 3A-like (without C2 domains) (Rph3al), mRNA.
NM_013016	0.011918091	Rattus norvegicus Protein tyrosine phosphatase, non-receptor type substrate 1 (SHP substrate 1) (Ptprns1), mRNA.
218402_Rn	0.011717513	Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 2 (Psma2), mRNA.
216197_Rn	0.01148805	Rattus norvegicus protein disulfide isomerase related protein (calcium-binding protein, intestinal-related) (Etp70), mRNA.
NM_019383	0.01129882	Rattus norvegicus ATP synthase subunit d (Atp5jd), mRNA.
344123_Rn	0.01129682	Rattus norvegicus dynein, cytoplasmic, light chain 1 (Pin), mRNA.
217738_Rn	0.011253014	similar to Mouse, clone MGC:37328 IMAGE:4975621, mRNA, complete cds.
NM_031022	0.011249981	Rattus norvegicus membrane-spanning proteoglycan NG2 (Cspg4), mRNA.
NM_017154	0.011235599	Rattus norvegicus xanthine dehydrogenase (Xdh), mRNA.
NM_033235	0.011047646	Rattus norvegicus Malate dehydrogenase-like enzyme (Mdhl), mRNA.
217910_Rn	0.011012779	Rattus norvegicus karyopherin (importin) alpha 2 (Kpna2), mRNA.
NM_012618	0.011011304	Rattus norvegicus S100 calcium-binding protein A4 (S100a4), mRNA.
205623_Rn	0.011007021	similar to Mouse piebald deletion region section 2 of 11 of the complete sequence.
NM_017152	0.010973397	Rattus norvegicus ribosomal protein S17 (Rps17), mRNA.
NM_017087	0.010899363	Rattus norvegicus biglycan (Bgn), mRNA.
323889_Rn	0.010882876	similar to Mouse, SH3-domain binding protein 2, clone MGC:11535 IMAGE:3967543, mRNA, complete cds.
A1234256	0.010870631	unknown function
282074_Rn	0.010699953	similar to CENP-E

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
BU759721	0.010617943	unknown function
219644_Rn	0.01041486	similar to mouse px19-like protein, clone MGC:30986 IMAGE:5251367, mRNA, complete cds.
NM_053959	0.010398549	Rattus norvegicus myc box dependent interacting protein 1 (Bin1), mRNA.
219704_Rn	0.0103239	similar to Mouse, RIKEN cDNA 1810074P22 gene, clone MGC:6997 IMAGE:3155195, mRNA, complete cds.
NM_013011	0.010153001	Rattus norvegicus Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta polypeptide (Ywhaz), mRNA.
AI764088	0.010143834	similar to Translation of nuc:AK02118_1 Mus musculus ES cells cDNA, RIKEN full-length enriched library, clone:C330006B10, full insert sequence; putative. score=1.57e-43
204964_Rn	0.010142466	unknown function
293402_Rn	0.010109723	similar to Mouse, midnolin, clone MGC:28080 IMAGE:3710931, mRNA, complete cds.
296464_Rn	0.010047228	Rattus norvegicus protein tyrosine phosphatase, non-receptor type substrate 1 (Ptprns1), mRNA.
326551_Rn	0.009996103	similar to data source:SPTR, source key:P56556, evidence:ISS~homolog to NADH-UBIQUINONE OXIDOREDUCTASE B14 SUBUNIT (EC 1.6.5.3) (EC 1.6.99.3) (COMPLEX I-B14) (CI-B14)~putative
BI277487	0.00996125	similar to Protein:NP_056550 damage specific DNA binding protein 1 (127 kDa) [Mus musculus]. score=9.375e-54
298335_Rn	0.009955471	unknown function
CA3333998	0.00993364	unknown function
280411_Rn	0.00992095	similar to Mouse Ig-like receptor PIR-A4 (6M7) mRNA, complete cds.
219577_Rn	0.009831183	similar to Mouse, clone IMAGE:3500261, mRNA, partial cds.
NM_053867	0.009787057	Rattus norvegicus tumor protein, translationally-controlled 1 (Tp57), mRNA.
NM_013220	0.009684891	Rattus norvegicus ankyrin-like repeat protein (Alrp), mRNA.
NM_019195	0.009615304	Rattus norvegicus integrin-associated protein (Cd47), mRNA.
BQ782938	0.00953195	Rat mRNA for ribosomal protein S27.
205290_Rn	0.009391507	similar to Mouse, RIKEN cDNA 2610041P16 gene, clone MGC:11643 IMAGE:3597862, mRNA, complete cds.
NM_030835	0.009371072	Rattus norvegicus ribosome associated membrane protein 4 (RAMP4), mRNA.
205973_Rn	0.009293626	Rattus norvegicus cathepsin K (Ctsk), mRNA.
NM_012605	0.009255043	Rattus norvegicus Myosin, light polypeptide 2, alkali; ventricular, skeletal, slow (Myl2), mRNA.
293672_Rn	0.009191981	Rat clone RP31-202M22 strain Brown Norway, complete sequence.
A1233144	0.009179013	unknown function
412096_Rn	0.009168671	Rattus norvegicus thymosin beta-4 (Tmsb4x), mRNA. GCGEST:BG664919
BF558629	0.009139426	unknown function

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_022506	0.009122465	Rattus norvegicus ribosomal protein L31 (Rpl31), mRNA.
218595_Rn	0.008984663	Rattus norvegicus procollagen C-proteinase enhancer protein (Pcolce), mRNA.
NM_013059	0.008935173	Rattus norvegicus alkaline phosphatase, tissue-nonspecific (Alpl), mRNA.
NM_012562	0.008883405	Rattus norvegicus Fucosidase, alpha-L-1, tissue (Fuca), mRNA.
219309_Rn	0.008791973	Rat heme oxygenase gene, complete cds.
294599_Rn	0.008769885	similar to Translation of nuc:X81624_1. M.auratus mRNA for Cl1 protein (Cl1-1). score=1.722e-12
201267_Rn	0.008744422	similar to Mouse mRNA for eIF3 p66, complete cds.
NM_017160	0.008735854	Rattus norvegicus ribosomal protein S6 (Rps6), mRNA.
220048_Rn	0.008725565	similar to Mouse lysosomal thiol reductase IP30 precursor, mRNA, partial cds.
223052_Rn	0.00868081	Rattus norvegicus Triosephosphate isomerase 1 (Tpi1), mRNA.
216538_Rn	0.008673028	Rattus norvegicus mRNA for NAD+-specific isocitrate dehydrogenase b-subunit, partial cds.
205140_Rn	0.008664165	similar to mouse hypothetical protein MGC3133, clone MGC:11596 IMAGE:3965951, mRNA, complete cds.
343399_Rn	0.00855704	similar to Mouse, clone MGC:11811 IMAGE:3595770, mRNA, complete cds.
220056_Rn	0.008431563	Rattus norvegicus Subtilisin - like endoprotease (Pace4), mRNA.
295525_Rn	0.008395076	Rattus norvegicus ATP-binding cassette, sub-family G (WHITE), member 1 (Abcg1), mRNA.
NM_022799	0.008294507	Rattus norvegicus nuclear ubiquitous casein kinase 2 (Nucks), mRNA.
221024_Rn	0.008263018	similar to Mouse, telomerase binding protein, p23, clone MGC:5681 IMAGE:3489418, mRNA, complete cds.
AI577060	0.008235996	unknown function
294679_Rn	0.008203782	unknown function
294570_Rn	0.008158666	Rattus norvegicus homocysteine respondent protein HCYP2 (Hcyp2), mRNA.
296547_Rn	0.00813859	similar to mouse replication factor C (activator 1) 3 (38kD), clone MGC:25594 IMAGE:4015134, mRNA, complete cds.
AA945587	0.008038106	unknown function
218472_Rn	0.007935005	Rattus norvegicus mRNA for collagen alpha 1 type XI.
CB545815	0.007634944	similar to Translation of nuc:AY049776_1 Homo sapiens unr-interacting protein (UNRIP) mRNA, complete cds. score=3.557e-33
NM_053590	0.007611677	Rattus norvegicus proteasome (prosome, macropain) subunit, beta type 1 (Psmb1), mRNA.
297802_Rn	0.007611231	unknown function
NM_019222	0.007608618	Rattus norvegicus coronin, actin-binding protein, 1B (Coro1b), mRNA.
298647_Rn	0.00758564	Rattus norvegicus collagen triple helix repeat containing 1 (Cthrc1), mRNA.
200080_Rn	0.007544825	Rattus norvegicus hsp86 gene for heat shock protein 86.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
217105_Rn	0.007480593	Rattus norvegicus ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit b, isoform 1 (Atp5f1), mRNA.
223206_Rn	0.007436423	similar to Mouse AMD1 gene for S-adenosylmethionine decarboxylase, complete cds.
BF415205	0.00735195	Rat mRNA fragment for cardiac actin.
NM_017166	0.007308496	Rattus norvegicus stathmin 1 (Stmn1), mRNA.
219673_Rn	0.007258145	Rattus norvegicus ribosomal protein S23 (Rps23), mRNA.
220755_Rn	0.007242171	similar to Hypothetical protein F54A3.5
223020_Rn	0.007214581	similar to Mouse mRNA for sid23p, complete cds.
343608_Rn	0.007146423	Rattus norvegicus ATP synthase, H ⁺ transporting, mitochondrial F1 complex, epsilon subunit (Atp5e), mRNA.
NM_022699	0.007124181	Rattus norvegicus ribosomal protein L30 (Rpl30), mRNA.
NM_031108	0.007040259	Rattus norvegicus ribosomal protein S9 (Rps9), mRNA.
NM_017138	0.007022981	Rattus norvegicus laminin receptor 1 (67kD, ribosomal protein SA) (Lamr1), mRNA.
NM_053605	0.007013728	Rattus norvegicus sphingomyelin phosphodiesterase 3, neutral (Smnpd3), mRNA.
206087_Rn	0.007013086	Rattus norvegicus F-actin binding protein b-Nexilin mRNA, complete cds.
AA901338	0.006962944	similar to Translation of nuc:X73836_1 O.cuniculus mRNA for eukaryotic initiation factor 2 beta (eIF-2 beta). score=9.897e-75
200835_Rn	0.006895106	Rattus norvegicus cytochrome c oxidase, subunit Va (Cox5a), mRNA.
BF419904	0.00687554	R.norvegicus mRNA for parathyroid hormone regulated sequence (92bp).
296501_Rn	0.006873167	similar to Mouse, Rac GTPase-activating protein 1, clone MGC:11396 IMAGE:3602242, mRNA, complete cds.
AI409191	0.006872879	unknown function
200849_Rn	0.006816801	Rattus norvegicus Creatine kinase, muscle form (Ckm), mRNA.
204800_Rn	0.00679428	similar to Mouse forkhead-related transcription factor 1C (Foxp1c)mRNA, complete cds.
223267_Rn	0.00668658	Rattus norvegicus ribosomal protein S12 (Rps12), mRNA.
344269_Rn	0.006652106	Rattus norvegicus sodium-coupled citrate transporter (NaCT), mRNA.
NM_022536	0.006647137	Rattus norvegicus cyclophilin B (Ppib), mRNA.
220815_Rn	0.006584966	similar to Translation of nuc:AF403037_1 Mus musculus SPRY domain-containing SOCS box protein SSB-2 mRNA, complete cds. score=4.573e-62
CA509937	0.006405841	Rattus norvegicus guanine nucleotide binding protein gamma 10 subunit mRNA, partial cds.
220915_Rn	0.006390185	Rattus norvegicus signal peptidase complex (18kD) (Spc18), mRNA.
BQ204885	0.006378802	unknown function
BQ207103	0.00637126	unknown function

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
CB547489	0.006297374	similar to Translation of nuc:AB067489_1 Homo sapiens mRNA for KIAA1902 protein, partial cds; Start codon is not identified.. score=3.858e-51
220886_Rn	0.006286905	Rattus norvegicus proteasome (prosome, macropain) subunit, alpha type 5 (Psma5), mRNA.
BQ207888	0.00627139	similar to Translation of nuc:AK003540_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110007H07, full insert sequence; putative. score=4.746e-14
222896_Rn	0.00622519	Rattus norvegicus CD164 antigen (Cd164), mRNA.
219437_Rn	0.006223531	similar to Mouse cysteine-rich intestinal protein (CRIP) mRNA, complete cds.
NM_022521	0.006182414	Rattus norvegicus ornithine aminotransferase (Oat), mRNA.
NM_031000	0.006168032	Rattus norvegicus aldo-keto reductase family 1, member A1 (Akr1a1), mRNA.
BF558699	0.00612421	unknown function
218711_Rn	0.006051737	Rattus norvegicus arginase 1, liver (Arg1), mRNA.
223247_Rn	0.006034867	Rat mRNA for ribosomal protein S16.
AI599332	0.00602388	unknown function
216375_Rn	0.005949521	Rat mRNA for epithelial membrane protein-1.
229336_Rn	0.005945782	similar to Mouse collagenous repeat-containing sequence of 26kDa protein (CORS26) mRNA, complete cds.
NM_053598	0.005943352	Rattus norvegicus diphosphoinositol polyphosphate phosphohydrolase type II (Nudt4), mRNA.
NM_017153	0.005912168	Rattus norvegicus ribosomal protein S3a (Rps3a), mRNA.
201835_Rn	0.005879829	Rattus norvegicus activating transcription factor ATF-4 (Atf4), mRNA.
Y16641_1	0.005831339	Rattus norvegicus mRNA for hnRNP protein, partial.
220184_Rn	0.00580148	Rattus norvegicus lectin, galactose binding, soluble 1 (Lgals1), mRNA.
NM_053610	0.005768097	Rattus norvegicus peroxiredoxin 6 (Prdx6), mRNA.
293162_Rn	0.00576711	similar to hypothetical protein FLJ13236
217954_Rn	0.005765612	similar to Mouse fibroblast growth factor inducible gene 14 (Fin14) mRNA, complete cds.
202298_Rn	0.005695825	similar to Translation of nuc:BC031262_1 Homo sapiens, Similar to catenin (cadherin-associated protein), alpha 2, clone MGC:39727 IMAGE:5274351, mRNA, complete cds. score=7.84e-45
200200_Rn	0.005682601	similar to Protein:NP_006734 RNA binding motif protein 3 [Homo sapiens]. score=2.622e-16
221477_Rn	0.005665553	Rattus norvegicus peroxiredoxin 1 (Prdx1), mRNA.
216044_Rn	0.005637257	similar to Mouse, signal recognition particle 14 kDa (homologous Alu RNA binding protein), clone MGC:36171 IMAGE:5353575, mRNA, complete cds.
222534_Rn	0.005630136	similar to Mouse, clone IMAGE:3583970, mRNA, partial cds.
201560_Rn	0.005584706	similar to Translation of nuc:AK002214_1 Mus musculus adult male kidney cDNA, RIKEN full-length enriched library, clone:0610005A19, full insert sequence; putative. score=1.142e-28

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
BQ209028	0.005550304	unknown function
BQ208015	0.005545714	similar to Protein:NP_081121 peptidylprolyl isomerase (cyclophilin)-like 1 [Mus musculus]. score=9.871e-82
222081_Rn	0.00546514	R.rattus mRNA for ribosomal protein L8.
435254_Rn	0.005459533	unknown function
203776_Rn	0.005216471	Rattus norvegicus glycoprotein (transmembrane) nmb (Gpnmb), mRNA.
215841_Rn	0.005188832	similar to Mouse DNA sequence from clone RP23-406B13 on chromosome 4, complete sequence.
NM_053602	0.005150686	Rattus norvegicus ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6 (Atp5j), mRNA.
226208_Rn	0.005104805	similar to Mouse Strain C57BL6/J chromosome 5 BAC, RP23-61K9, Complete Sequence, complete sequence.
220656_Rn	0.005095019	Rattus norvegicus ribosomal protein L10a (Rpl10a), mRNA.
NM_022515	0.005048616	Rattus norvegicus ribosomal protein L24 (Rpl24), mRNA.
202815_Rn	0.005042476	similar to Mouse, RIKEN cDNA 1300011C24 gene, clone MGC:7169 IMAGE:3257323, mRNA, complete cds.
200476_Rn	0.004998189	Rattus norvegicus Calmodulin 2 (phosphorylase kinase, delta) (Calm2), mRNA.
208250_Rn	0.004980438	Rattus norvegicus interferon gamma receptor (Ifngr), mRNA.
206584_Rn	0.004941247	Rattus norvegicus smooth muscle cell LIM protein (SmLIM) mRNA, complete cds.
NM_019299	0.004920952	Rattus norvegicus clathrin, heavy polypeptide (Hc) (Cltc), mRNA.
347198_Rn	0.004904098	similar to Mouse, RIKEN cDNA 2310045A07 gene, clone MGC:41044 IMAGE:1397989, mRNA, complete cds.
221755_Rn	0.004802136	unknown function
296765_Rn	0.004747164	similar to Mouse Nedd4 WW domain-binding protein 4 mRNA, partial cds.
199779_Rn	0.004745905	Rat mRNA for the cysteine proteinase inhibitor cystatin C.
218904_Rn	0.00470795	similar to KIAA0782 protein
M92059.1	0.004707921	Rattus norvegicus adipsin mRNA sequence.
222657_Rn	0.00469367	similar to Mouse, RIKEN cDNA 9130413I22 gene, clone MGC:7196 IMAGE:3482091, mRNA, complete cds.
AA925693	0.004692767	similar to gi 202653 gb J00691 RATACCYB Rat cytoplasmic beta-actin gene, complete cds, mRNA sequence.
385033_Rn	0.004658736	Rattus norvegicus high mobility group box 2 (Hmgb2), mRNA.
221686_Rn	0.004658001	Rattus norvegicus calponin 3, acidic (Cnn3), mRNA.
203358_Rn	0.004573301	Rat c-fos mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
222773_Rn	0.004501696	similar to data source:SPTR, source key:O14521, evidence:ISS~homolog to SUCCINATE DEHYDROGENASE [UBIQUINONE] CYTOCHROME B SMALL SUBUNIT, MITOCHONDRIAL PRECURSOR (CYBS)~putative
221601_Rn	0.004468579	Rattus norvegicus Fibronectin 1 (Fn1), mRNA.
218034_Rn	0.004467934	Rat collagen XVIII mRNA, partial cds.
220565_Rn	0.004376637	similar to Translation of nuc:IAK003540_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110007H07, full insert sequence; putative. score=4.746e-14
NM_017148	0.004371136	Rattus norvegicus cysteine rich protein 1 (Csp1), mRNA.
298460_Rn	0.004289644	Rattus norvegicus Early growth response 1 (Egr1), mRNA.
218622_Rn	0.004279089	similar to Mouse pigment epithelium-derived factor (PEDF) mRNA, complete cds.
220301_Rn	0.004196862	similar to mouse RAB31, member RAS oncogene family, clone MGCG:7715 IMAGE:3497938, mRNA, complete cds.
CB545139	0.004114784	similar to Translation of nuc:S57132_1 COL16A1=type XVI collagen alpha 1 chain [human, placenta, mRNA Partial, 3720 nt]. This sequence comes from Fig. 2; alpha 1 (XVI). score=2.978e-26
NM_031570	0.004114166	Rattus norvegicus ribosomal protein S7 (Rps7), mRNA.
205016_Rn	0.004009854	similar to data source:S PTR, source key:O95168, evidence:ISS~homolog to NADH-UBIQUINONE OXIDOREDUCTASE B15 SUBUNIT (EC 1.6.5.3) (EC 1.6.99.3) (COMPLEX I-B15) (CI-B15)~putative Rat troponin-c mRNA.
430014_Rn	0.00399305	{clone E592/594, estrogen induced gene} [rats, Sprague-Dawley hypothalamus, mRNA Partial, 183 nt].
CA508092	0.003951845	Rattus norvegicus high-affinity immunoglobulin gamma Fc receptor I mRNA, partial cds.
BQ189985	0.003705459	Rattus norvegicus Farnesyl diphosphate synthase (Fdps), mRNA.
199948_Rn	0.003612093	similar to Protein:NP_080731 RIKEN cDNA 2510049i19 gene [Mus musculus]. score=5.034e-32
221395_Rn	0.003586152	UI-R-A1-eo-f-12-0-UI.s1 UI-R-A1 Rattus norvegicus cDNA clone UI-R-A1-eo-f-12-0-UI 3 similar to gi 2918669 dbj C86712 C86712 Mus musculus fertilized egg cDNA 3-end sequence, clone J0231G01, mRNA sequence.
413406_Rn	0.003583909	R.rattus mRNA for dermatan sulfate proteoglycan-II (decorin).
322873_Rn	0.003526308	unknown function
AA858954	0.003512611	Rattus norvegicus Small inducible gene JE (Scya2), mRNA.
292212_Rn	0.003464036	U75926.1 0.003405713 Rattus norvegicus cytochrome oxidase subunit VIIa mRNA, 5 untranslated region, partial sequence.
BE107038	0.003379084	unknown function
322974_Rn	0.003298478	similar to Mouse alcohol dehydrogenase Pan1b mRNA, complete cds.
221393_Rn	0.003285181	similar to Protein:NP_033145 Sin3-associated polypeptide 18 [Mus musculus]. score=6.753e-59
NM_0222179	0.003145482	Rattus norvegicus Hexokinase 3 (Hk3), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
AW525201	0.003106061	unknown function
201953_Rn	0.003101484	similar to Mouse ElA (Serpib1) mRNA, complete cds.
218829_Rn	0.00305058	Rat mRNA for ribosomal protein S25.
NM_022514	0.003049935	Rattus norvegicus ribosomal protein L27 (Rpl27), mRNA.
287902_Rn	0.003011509	similar to Mouse, asporin, clone MGC:41375 IMAGE:1365428, mRNA, complete cds.
NM_022510	0.002978847	Rattus norvegicus ribosomal protein L4 (Rpl4), mRNA.
BE105985	0.002968754	unknown function
NM_013091	0.002964753	Rattus norvegicus Tumor necrosis factor receptor (Tnfr1), mRNA.
413546_Rn	0.002951621	Rattus norvegicus mitochondrial voltage dependent anion channel 3 (Vdac3), mRNA.
NM_134353	0.002932418	Rattus norvegicus poly(A) binding protein, cytoplasmic 1 (Pabpc1), mRNA.
222544_Rn	0.00292739	Rattus norvegicus Cholesterol esterase (pancreatic) (Lipa), mRNA.
AA858647	0.002913092	unknown function
CA509950	0.002889357	Rattus sp. 3 UTR.
222629_Rn	0.002799082	Rattus norvegicus embigin (Emb), mRNA.
413656_Rn	0.00276803	Rattus norvegicus heterogeneous nuclear ribonucleoprotein A1 (Hnnpa1), mRNA.
217559_Rn	0.002739312	similar to Mouse mRNA for non-histone chromosomal protein HMG-14.
217519_Rn	0.002657967	similar to Mouse, clone MGC:37911 IMAGE:5102315, mRNA, complete cds.
219058_Rn	0.002641218	Rattus norvegicus S100 calcium-binding protein A9 (calgranulin B) (S100a9), mRNA.
NM_021264	0.002570467	Rattus norvegicus ribosomal protein L35a (Rpl35a), mRNA.
AA859114	0.002550828	similar to Protein:NP_08712 RIKEN cDNA 1810054O13 [Mus musculus]. score=6.377e-40
NM_054006	0.002530758	Rattus norvegicus unr protein (unr), mRNA.
CB545131	0.00250167	similar to Protein:NP_055392 bromodomain containing protein 1; BR140-like gene [Homo sapiens]. score=7.563e-63
CB544878	0.002466638	unknown function
219818_Rn	0.002446019	similar to Mouse spermidine/spermine N1-acetyltransferase (SSAT) mRNA, complete cds.
220191_Rn	0.002428659	Rattus norvegicus Annexin V (Anx5), mRNA.
220260_Rn	0.002427494	Rattus norvegicus fatty acid binding protein 5, epidermal (Fabp5), mRNA.
NM_022597	0.002421296	Rattus norvegicus cathepsin B (Ctsb), mRNA.
NM_053467	0.002307612	Rattus norvegicus integral membrane protein Tmp21-1 (p23) (Tmp21), mRNA.
CB548315	0.00230335	similar to Protein:NP_084515 ATP-binding cassette, subfamily G, member 3 [Mus musculus]. score=6.816e-62
205807_Rn	0.002255329	Rattus norvegicus ribosomal protein L24 (Rpl24), mRNA. GCGEST:BG671633
200663_Rn	0.002233586	Rattus norvegicus vimentin (Vim), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
222095_Rn	0.0022233554	Rattus norvegicus glutathione peroxidase (GSH-PO) mRNA, complete cds.
231072_Rn	0.002207388	similar to Mouse, clone IMAGE:3589736, mRNA, partial cds.
217929_Rn	0.002188342	similar to Mouse mRNA for reticulocalbin, complete cds.
NM_012881	0.002185717	Rattus norvegicus secreted phosphoprotein 1 (Spp1), mRNA.
295729_Rn	0.002169041	Rat thymocyte L-CA (leukocyte common antigen) mRNA, 3 flank.
NM_013026	0.002149256	Rattus norvegicus Syndecan (Synd1), mRNA.
223588_Rn	0.002099154	Rattus norvegicus macrophage galactose N-acetyl-galactosamine specific lectin (Mgl), mRNA.
NM_012904	0.002044185	Rattus norvegicus Annexin 1 (p35) (Lipocortin 1) (Anx1), mRNA.
CA505434	0.00200634	unknown function
NM_053439	0.001973018	Rattus norvegicus RAN, member RAS oncogene family (Ran), mRNA.
287302_Rn	0.001964941	Rat mitochondrial H+-ATP synthase alpha subunit mRNA, complete cds.
NM_031629	0.001964755	Rattus norvegicus proteasome (prosome, macropain) subunit, beta type 4 (Psmb4), mRNA.
218823_Rn	0.001949221	similar to Mouse, clone MGC:28751 IMAGE:4482756, mRNA, complete cds.
AA900381	0.001917056	unknown function
218353_Rn	0.001897489	Rattus norvegicus Ornithine decarboxylase antizyme (Oaz), mRNA.
221213_Rn	0.001892114	Rattus norvegicus Syndecan 1 (Sdc1), mRNA.
NM_022697	0.001884179	Rattus norvegicus ribosomal protein L28 (Rpl28), mRNA.
292230_Rn	0.001862641	Rattus norvegicus dual specificity phosphatase 6 (Dusp6), mRNA.
199660_Rn	0.001853378	Rattus norvegicus ribosomal protein S3a (Rps3a), mRNA.
NM_019289	0.001842916	Rattus norvegicus Actin-related protein complex 1b (Arpc1b), mRNA.
221438_Rn	0.001776963	similar to mouse RIKEN cDNA 2400003B06 gene, clone MGC:7860 IMAGE:3501295, mRNA, complete cds.
298020_Rn	0.001729723	similar to Mouse homeobox gene Prx2 mRNA.
296381_Rn	0.001721575	similar to RAB5 interacting protein 3
206323_Rn	0.001701615	Rattus norvegicus integral membrane protein Tmp21-I (p23) (Tmp21), mRNA.
AI411057	0.001689435	unknown function
293007_Rn	0.001653896	Rattus norvegicus serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (Serpine1), mRNA.
292104_Rn	0.0016529	similar to Translation of nuc:BC032959_1 Mus musculus, triggering receptor expressed on myeloid cells 2a, clone MGC:40999 IMAGE:1348066, mRNA, complete cds. score=2.498e-69
217258_Rn	0.001645597	Rattus norvegicus ribosomal protein L27 (Rpl27), mRNA.
CB547219	0.001637272	unknown function
221942_Rn	0.001627929	Rattus norvegicus Cd63 antigen (Cd63), mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_012876	0.001623056	Rattus norvegicus Ribosomal protein S29 (Rps29), mRNA.
222590_Rn	0.001583154	Rattus norvegicus ribosomal protein L36 (Rpl36), mRNA.
NM_017066	0.00155346	Rattus norvegicus Pleiotrophin (Heparine binding factor, Hbnf, in the mouse) (Ptn), mRNA.
NM_053291	0.00146793	Rattus norvegicus Phosphoglycerate kinase 1 (Pgk), mRNA.
CA508481	0.001413241	unknown function
199927_Rn	0.001413027	Rattus norvegicus H3 histone, family 3B (H3f3b), mRNA.
NM_138828	0.001389949	Rattus norvegicus Apolipoprotein E, (Apoe), mRNA.
219224_Rn	0.001388485	Rattus norvegicus Diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein) (Dbi), mRNA.
273885_Rn	0.001385987	Rattus norvegicus complement component 5, receptor 1 (C5r1), mRNA.
CB606336	0.001381343	unknown function
200661_Rn	0.001376264	similar to Mouse, clone MGC:6469 IMAGE:2631779, mRNA, complete cds.
NM_057144	0.001259786	Rattus norvegicus cysteine-rich protein 3 (Csrp3), mRNA.
NM_031105	0.00122689	Rattus norvegicus large subunit ribosomal protein L36a (Rpl36a), mRNA.
NM_080698	0.001218503	Rattus norvegicus fibromodulin (Fmod), mRNA.
221153_Rn	0.001217213	Rattus norvegicus mRNA for cathepsin Y, partial cds.
222608_Rn	0.001205378	similar to Mouse DNA sequence from clone RP24-144L11 on chromosome 4, complete sequence.
NM_012771	0.001191971	Rattus norvegicus Lysozyme (Lyz), mRNA.
294206_Rn	0.001163216	similar to Mouse strain C57BL/6J baslin mRNA, complete cds.
NM_012512	0.001117141	Rattus norvegicus Beta-2-microglobulin (B2m), mRNA.
NM_134334	0.001087013	Rattus norvegicus cathepsin D (Ctsd), mRNA.
222867_Rn	0.001081208	Rattus norvegicus Benzodiazepin receptor (peripheral) (Bzrp), mRNA.
U57362.1	0.001077252	Rattus norvegicus collagen XII alpha 1 (Col12a1) mRNA, partial cds.
NM_031103	0.001070446	Rattus norvegicus ribosomal protein L19 (Rpl19), mRNA.
208320_Rn	0.001030969	similar to evidence:NAS~hypothetical protein~putative
217893_Rn	0.001006907	Rattus norvegicus heat shock 70kD protein 5 (Hspa5), mRNA.
NM_012998	0.001002391	Rattus norvegicus Protein disulfide isomerase (Prolyl 4-hydroxylase, beta polypeptide) (P4hb), mRNA.
343323_Rn	9.61E-04	similar to Mouse FKBP65 binding protein mRNA, complete cds.
NM_031687	9.41E-04	Rattus norvegicus ubiquitin A-52 residue ribosomal protein fusion product 1 (Uba52), mRNA.
BQ200384	9.00E-04	unknown function
NM_031114	8.58E-04	Rattus norvegicus S-100 related protein, clone 42C (S100A10), mRNA.
199395_Rn	8.56E-04	Rattus norvegicus ribosomal protein S11 (Rps11), mRNA.
NM_057114	8.39E-04	Rattus norvegicus peroxiredoxin 1 (Prdx1), mRNA.

Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing Greater than 2-Fold UP-regulated

Gene ID	P-value	Gene Function
NM_012587	7.89E-04	Rattus norvegicus integrin binding sialoprotein (Ibsp), mRNA.
219516_Rn	7.81E-04	Rattus norvegicus calcium binding protein A6 (calcyclin) (S100a6), mRNA.
217733_Rn	7.67E-04	similar to Mouse, RIKEN cDNA 1300019i03 gene, clone MGC:6625 IMAGE:3491528, mRNA, complete cds.
NM_053843	7.24E-04	Rattus norvegicus Fc receptor, IgG, low affinity III (Fcgr3), mRNA.
413868_Rn	7.07E-04	Rattus norvegicus ribosomal protein S8 (Rps8), mRNA.
347975_Rn	6.64E-04	similar to Protein:NP_077751 testis expressed gene 189 [Mus musculus]. score=2.117e-116
296414_Rn	6.62E-04	unknown function
NM_012992	6.56E-04	Rattus norvegicus Nucleoplasmmin-related protein (Nuclear protein B23 (Npm1), mRNA.
CB548041	6.35E-04	similar to Protein:NP_663384 similar to cutlet [Mus musculus]. score=1.492e-20
BQ210920	6.26E-04	unknown function
NM_019360	6.24E-04	Rattus norvegicus cytochrome oxidase subunit Vlc (Cox6c), mRNA.
221680_Rn	6.21E-04	Rattus norvegicus ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit (oligomycin sensitivity conferring protein) (Atp5o), mRNA.
NM_031101	5.97E-04	Rattus norvegicus ribosomal protein L13 (Rpl13), mRNA.
200356_Rn	5.85E-04	Rattus norvegicus alpha-tubulin (Tuba1), mRNA.
221392_Rn	5.83E-04	Rattus norvegicus Matrix Gla protein (Mgp), mRNA.
412899_Rn	5.81E-04	similar to Mouse Strain C57BL6/J Chromosome X BAC, RP23-64A09, Complete Sequence, complete sequence.
NM_131914	5.56E-04	Rattus norvegicus caveolin 2 (Cav2), mRNA.
NM_033539	5.52E-04	Rattus norvegicus eukaryotic translation elongation factor 1 alpha 2 (Eef1a2), mRNA.
CA507265	4.85E-04	unknown function
208422_Rn	4.53E-04	Rattus norvegicus alanyl (membrane) aminopeptidase (Anpep), mRNA.
348054_Rn	4.53E-04	Rattus norvegicus gas-5 growth arrest homolog non-translated mRNA sequence.
216413_Rn	4.32E-04	Rattus norvegicus ribosomal protein L6 (Rpl6), mRNA.
NM_053982	4.09E-04	Rattus norvegicus ribosomal protein S15a (Rps15a), mRNA.
201846_Rn	3.73E-04	Rattus norvegicus ribosomal protein L10 (Rpl10), mRNA. GCGEST:BG671435
NM_022674	3.70E-04	Rattus norvegicus H2A histone family, member Z (H2afz), mRNA.
206738_Rn	3.49E-04	Rat metallothionein-2 and metallothionein-1 genes, complete cds.
BQ191086	3.39E-04	similar to Protein:NP_079785 RIKEN cDNA 231008M10 gene [Mus musculus]. score=1.467e-63
AW526982	3.37E-04	unknown function
430016_Rn	2.60E-04	Rat mRNA for ribosomal protein S19.
NM_022226	2.50E-04	Rattus norvegicus protease, cysteine, 1 (legumain) (Prsc1), mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
217441_Rn	2.31E-04	similar to Mouse, Deleted in split-hand/split-foot 1 region, clone MGC:31011 IMAGE:5251089, mRNA, complete cds.
199802_Rn	2.23E-04	Rattus norvegicus ribosomal protein L21 (Rpl21), mRNA.
BQ205045	1.72E-04	unknown function
215832_Rn	1.16E-04	similar to Mouse, RIKEN cDNA 3110037K17 gene, clone MGC:41407 IMAGE:1496162, mRNA, complete cds.
NM_031099	1.07E-04	Rattus norvegicus ribosomal protein L5 (Rpl15), mRNA.
NM_031106	8.95E-05	Rattus norvegicus ribosomal protein L37 (Rpl37), mRNA.
NM_013156	6.41E-05	Rattus norvegicus Cathepsin L (Ctsl), mRNA.
NM_031111	4.79E-05	Rattus norvegicus ribosomal protein S21 (Rps21), mRNA.
199774_Rn	4.22E-05	Rat mRNA for ribosomal protein L9.
218377_Rn	3.17E-05	Rattus norvegicus large subunit ribosomal protein L36a (Rpl36a), mRNA.

**Table 1: Significant 2-Fold Gene Expression Changes at 3 Days Fracture Healing
Greater than 2-Fold DOWN-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
200837_Rn	0.047027304	Rattus norvegicus defensin NP-2 precursor (LOC286995), mRNA.
NM_017055	0.036387031	Rattus norvegicus Transferrin (Tf), mRNA.
208234_Rn	0.013435548	Rattus norvegicus coronin, actin binding protein 1A (Coro1a), mRNA.

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
221163_Rn	0.04976948	unknown function
298811_Rn	0.049730247	Rattus norvegicus Smhs1 protein (Smhs1), mRNA.
NM_031007	0.049687102	Rattus norvegicus adenylyl cyclase 2 (Adcy2), mRNA.
207909_Rn	0.049383069	unknown function
278459_Rn	0.048583013	similar to Mouse Six1 mRNA.
BF416270	0.048208788	unknown function
NM_012880	0.047743033	Rattus norvegicus Superoxide dimutase 3 (Sod3), mRNA.
384905_Rn	0.046817862	similar to Protein:NP_075875 28kD interferon alpha responsive protein [Mus musculus]. score=3.208e-45
BF416262	0.046277827	unknown function
CB545303	0.045324534	similar to Translation of nuc:AF454755_1 Mus musculus vitrin (Vit) mRNA, complete cds; vitreous protein. score=8.359e-53
BQ209192	0.044374361	similar to Mouse brain cDNA, clone MNCb-3527, similar to AF220152 TACC2 (Human).
222428_Rn	0.044332706	unknown function
BF546421	0.043819681	unknown function
CB546887	0.043699317	similar to Translation of nuc:AF048695_1 Mus musculus type I alpha phosphatidylinositol-4-phosphate 5-kinase variant (Pip5ka) mRNA, complete cds. score=2.799e-60
NM_031016	0.041806517	Rattus norvegicus muscarinic receptor m2 (Chrm2), mRNA.
216328_Rn	0.041642695	similar to Mouse nebulin mRNA, partial cds.
219340_Rn	0.041517835	Rattus norvegicus fibroblast growth factor 13 (Fgf13), mRNA.
298020_Rn	0.041331433	similar to Mouse homeobox gene Prx2 mRNA.
298291_Rn	0.041096369	similar to Mouse chromosome 11a2 clone rp21-493n6 strain 129S6/SvEvTac, complete sequence.
CB545321	0.040977313	similar to Protein:NP_032635 matrix metalloproteinase 15; Membrane type 2-MMP [Mus musculus]. score=5.754e-39
409153_Rn	0.040895411	similar to data source:SPTR, source key:P70193, evidence:ISS~putative~similar to MEMBRANE GLYCOPROTEIN
CB544481	0.039919633	similar to Protein:NP_032623 matrix gamma-carboxyglutamate (gla) protein [Mus musculus]. score=2.363e-21
NM_013043	0.039496822	Rattus norvegicus Transforming growth factor beta stimulated clone 22 (Tgfb14), mRNA.
298157_Rn	0.039362131	similar to Mouse DNA sequence from clone RP23-206I14 on chromosome 2, complete sequence.
M20035_1	0.039342648	Rat prothymosin-alpha mRNA, complete cds.
414372_Rn	0.038847833	Rat clone RP31-249D7 strain Brown Norway, complete sequence.
384548_Rn	0.038617251	Rattus norvegicus 25 oligoadenylylate synthetase (Oas1), mRNA.

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
216692_Rn	0.037885779	similar to hypothetical protein
232193_Rn	0.037282673	similar to hypothetical protein
CA338396	0.036670827	similar to Protein:NP_077168 RIKEN cDNA 1110003H02 gene [Mus musculus]. score=2.884e-69
BF543297	0.036547921	unknown function
CB606202	0.035864013	similar to Protein:NP_036055 CD97 antigen [Mus musculus]. score=1.682e-21
BF555973	0.035776136	unknown function
AW531805	0.035544976	similar to Protein:NP_034631 interferon-induced protein with tetratricopeptide repeats 3 [Mus musculus]. score=5.227e-17
295703_Rn	0.035289026	similar to Mouse, clone MGC:29057 IMAGE:3673874, mRNA, complete cds.
AI009167	0.034326135	Rat adipocyte hormone-sensitive cAMP phosphodiesterase mRNA, complete cds.
AA858962	0.034087173	unknown function
295756_Rn	0.032958526	Rat retinol-binding protein (RBP) mRNA, partial cds.
AA963765	0.032631833	unknown function
288031_Rn	0.032406741	similar to Protein:NP_032786 osteoglycin [Mus musculus]. score=1.428e-105
284357_Rn	0.032365766	Rat mRNA for alternatively spliced smooth muscle myosin heavy chain (clone RAMHC21).
U44948_1	0.031995375	unknown function
218106_Rn	0.031554825	Rattus norvegicus smooth muscle cell LIM protein (SmLIM) mRNA, complete cds.
BI288713	0.031178518	Rattus norvegicus Transforming growth factor beta stimulated clone 22 (Tgfb1i4), mRNA.
252842_Rn	0.031099168	unknown function
343632_Rn	0.029970802	Rattus norvegicus a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS-1) (Adams1), mRNA.
220300_Rn	0.029530905	similar to Mouse interferon regulatory factor 7 (mirf7) mRNA, complete cds.
216084_Rn	0.029496484	Rat cAMP-dependent protein kinase inhibitor (PKI) mRNA, complete cds.
AI176675	0.029457862	unknown function
CB546846	0.029185395	similar to GCGPROT:O43147 KIAA0397 PROTEIN (FRAGMENT). score=2.625e-56
201856_Rn	0.02916694	Rat myoglobin mRNA, complete cds.
222856_Rn	0.028582738	similar to Protein:NP_036039 ubiquitin specific protease 18; ubiquitin specific protease 15 [Mus musculus]. score=5.093e-36
229344_Rn	0.028431925	Rattus norvegicus Myogenin factor 6 (herculin) (Myf6), mRNA.
AA946444	0.028148529	unknown function
BF281839	0.02796791	unknown function
NM_053381	0.027864753	Rattus norvegicus ATPase, (Na+)/K+ transporting, beta 4 polypeptide (Atp1b4), mRNA.

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
294187_Rn	0.027678647	unknown function
220377_Rn	0.027123665	similar to Mouse, FK506-binding protein 3 (25kD), clone MGC:6631 IMAGE:3492535, mRNA, complete cds.
218621_Rn	0.026765322	Rattus norvegicus Carnitine palmitoyltransferase 1 beta, muscle isoform (Cpt1b), mRNA.
221392_Rn	0.026728454	Rattus norvegicus Matrix Gla protein (Mgp), mRNA.
CA511442	0.02637318	unknown function
BE109129	0.025396623	unknown function
322818_Rn	0.024717226	similar to Mouse, clone IMAGE:3489640, mRNA.
298362_Rn	0.024141303	Rattus norvegicus cysteine rich protein 61 (Cyr61), mRNA.
219784_Rn	0.023404328	similar to Mouse cofilin isoform mRNA, complete cds.
BE0999435	0.023368666	unknown function
283221_Rn	0.023366021	similar to mouse RIKEN cDNA 4933425F03 gene, clone MGC:27633 IMAGE:4506472, mRNA, complete cds.
205548_Rn	0.023098558	similar to Mouse, matrilin 2, clone MGC:5875 IMAGE:3492881, mRNA, complete cds.
BF566263	0.022922933	similar to Translation of nuc:BC027795_1 Mus musculus, Similar to zinc finger protein 294, clone IMAGE:5251357, mRNA, partial cds, score=1.87e-90
AW525918	0.022394022	mRNA, partial cds, score=2.224e-100
251419_Rn	0.022361102	similar to Protein:NP_071710 secreted modular calcium-binding protein 2 [Mus musculus]. score=1.953e-70
221116_Rn	0.022359994	Rattus norvegicus FXYD domain-containing ion transport regulator 6 (Fxyd6), mRNA.
BQ209308	0.02221918	Rattus norvegicus partial mRNA for mono (ADP-ribosyl)transferase (ART3 gene).
221824_Rn	0.021755549	similar to Mouse, clone MGC:28735 IMAGE:4460992, mRNA, complete cds.
AA925141	0.021562998	similar to gi 2557199 gb AA6333985 AA6333985 ac33f04.s1 Stratagene hNT neuron (#937233) Homo sapiens cDNA clone 858271_3, mRNA sequence.
295799_Rn	0.020824167	Rattus norvegicus calcium channel, voltage-dependent, alpha2/delta subunit 1 (Cacna2), mRNA.
205857_Rn	0.020549915	0.020296265
CA338648	0.020296265	Rattus norvegicus delta-like homolog (Drosophila) (Dlk1), mRNA.
294332_Rn	0.019977612	unknown function
AA850867	0.019974476	similar to Mouse DNA sequence from clone RP23-113P22 on chromosome 4, complete sequence.
NM_130743	0.0199919763	similar to Protein:NP_036022 sarcoglycan, gamma (35kD dystrophin-associated glycoprotein) [Mus musculus]. score=3.114e-43
221410_Rn	0.019856963	Rattus norvegicus interferon, alpha-inducible protein 27-like (Ifi27l), mRNA.
AI112095	0.019425331	similar to Mouse gene for neural cell adhesion molecule 3 region exon b; NCAM-120 C-term.
220900_Rn	0.019352262	unknown function
200130_Rn	0.019296916	Rattus norvegicus troponin T2, cardiac (Tnnt2), mRNA.

**Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
384058_Rn	0.01894611	similar to Protein:NP_113578 upregulated during skeletal muscle growth 4; DNA segment, Chr 3, Brigham & Womens Genetics 1078 expressed [Mus musculus]. score=4.545e-12
BF401264	0.018038843	unknown function
NM_053395	0.017720716	Rattus norvegicus small muscle protein, X-linked (Smpx), mRNA.
298899_Rn	0.01726424	unknown function
199765_Rn	0.017038842	Rattus norvegicus troponin I, skeletal, fast 2 (Tnni2), mRNA.
206584_Rn	0.016975793	Rattus norvegicus smooth muscle cell LIM protein (SmLIM) mRNA, complete cds.
NM_016990	0.016731409	Rattus norvegicus Adducin 1, alpha (Add1), mRNA.
BQ211970	0.015887359	similar to Protein:NP_057921 tropomodulin 4 [Mus musculus]. score=2.081e-49
199469_Rn	0.015109729	Rattus norvegicus BCL2/adenovirus E1B 19 kDa-interacting protein 3, nuclear gene for mitochondrial product (Bnip3), mRNA.
218120_Rn	0.015103888	similar to amylo-1, 6-glucosidase/4-alpha-glucantransferase
206075_Rn	0.015062511	similar to mouse annexin A8, clone MGC:13875 IMAGE:4013266, mRNA, complete cds.
200473_Rn	0.014578753	Rattus norvegicus N-myc downstream-regulated gene 2 (Ndrg2), mRNA.
297341_Rn	0.014561725	similar to Translation of nuc:S71251_1 monocyte chemotactic protein-3 [mice, macrophage cell line WEHI-3, mRNA, 808 nt]; This sequence comes from Fig. 1; MCP-3; MarcFic protein. score=7.81e-42
221113_Rn	0.01456035	Rattus norvegicus small inducible cytokine B subfamily (Cys-X-Cys), member 10 (Scyb10), mRNA.
NM_019145	0.014553218	Rattus norvegicus cholinergic receptor, nicotinic, gamma polypeptide (Chrg), mRNA.
BI276352	0.014349707	similar to Protein:NP_112019 protein kinase C and casein kinase substrate in neurons 3 [Mus musculus]. score=3.547e-55
298983_Rn	0.014075765	similar to myopalladin
294630_Rn	0.013775547	similar to Mouse DNA sequence from clone RP23-391E6 on chromosome 4, complete sequence.
219145_Rn	0.012507577	similar to Mouse DNA sequence from clone RP23-141C15 on chromosome 4, complete sequence.
AI172305	0.012486349	similar to Translation of nuc:AK009124_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310003M01, full insert sequence; putative. score=8.841e-72
NM_022190	0.0123316201	Rattus norvegicus aggrecan 1 (Agc1), mRNA.
200201_Rn	0.012185791	Rattus norvegicus ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (Atp2a2), mRNA.
206087_Rn	0.011973302	Rattus norvegicus F-actin binding protein b-Nexilin mRNA, complete cds.
377134_Rn	0.011901885	similar to mouse a disintegrin and metalloprotease with thrombospondin motifs 1 (ADAMTS-1), clone IMAGE:3491991, mRNA, partial cds.
AI145097	0.011694643	unknown function

**Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
BQ782850	0.011464648	similar to Translation of nuc:AL_160153_1 Human DNA sequence from clone RP11-397O8 on chromosome 13 Contains the 3 end of the ITBGL1 (integrin, beta-like 1 (with EGF-like repeat domains)) gene, the 3 end of the FGF14 (fibroblast growth factor 14) gene, ESTs, STSs, and GSSs, complete sequence; match: proteins: Tr:O14549 Tr:Q9NPR0 Sw:P09055 Sw:P53712 Sw:P12607 Sw:P07228 Sw:P12606 Sw:P11835 Sw:P49134 Tr:O96444 Tr:O17494. score=7.802e-11
231197_Rn	0.011099532	similar to Mouse DNA sequence from clone RP23-381K21 on chromosome 1, complete sequence. Rattus norvegicus myc box dependent interacting protein 1 (Bin1), mRNA.
NM_053959	0.010933826	similar to Translation of nuc:BC028939_1 Mus musculus, Similar to PMS1 postmeiotic segregation increased 1 (S. cerevisiae), clone MGC:36491 IMAGE:5364251, mRNA, complete cds. score=2.133e-36
CB547248	0.010900596	similar to mouse MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C), clone MGC:25468 IMAGE:4481227, mRNA, complete cds.
296334_Rn	0.010333453	Rattus norvegicus ras-like protein (Tc10), mRNA.
NM_053522	0.01015523	unknown function
229083_Rn	0.009945898	similar to unnamed protein product
296736_Rn	0.00993046	Rat hypertrophied skeletal muscle protein FHL1c mRNA, complete cds.
216023_Rn	0.009809025	similar to Mouse chromosome 6 clone rp23-176m22 strain C57BL/6J, complete sequence.
218958_Rn	0.00966848	similar to Mouse, clone MGC:7583 IMAGE:3493553, mRNA, complete cds.
207673_Rn	0.009644362	Rat succinate-semialdehyde dehydrogenase (SSADH) mRNA, 3 end.
L34821_1	0.0095308	similar to Translation of nuc:AF026204_8 Caenorhabditis elegans cosmid C30E1. score=6.062e-09
CB547577	0.009434145	similar to TSP2=thrombospondin 2 [Mouse, Genomic, 2030 nt, segment 2 of 2].
298704_Rn	0.009261067	Rat angiotensin II type 1A receptor associated protein mRNA, complete cds.
298372_Rn	0.009118459	Rattus norvegicus selectin, lymphocyte (Sell), mRNA.
NM_019177	0.009110869	similar to Translation of nuc:S57132_1 COL16A1=type XVI collagen alpha 1 chain [human, placenta, mRNA Partial, 3720 nt]; This sequence comes from Fig. 2; alpha 1 (XVI). score=2.978e-26
CB545139	0.008801184	Rat ASM15 gene.
218107_Rn	0.008712971	norvegicus similar to DKKZP586C1619 protein [Homo sapiens] (LOC314675), mRNA.
218836_Rn	0.008475641	similar to GCGPROT:Q64220 THYROTROPIN RECEPTOR (FRAGMENT). score=3.134e-23
CB547020	0.008317261	similar to Protein:NP_598743 expressed sequence AI851155 [Mus musculus]. score=1.798e-52
CB545955	0.008303068	similar to Translation of nuc:AK009491_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:23:10024D23, full insert sequence; putative. score=4.186e-39
259991_Rn	0.008228418	R.norvegicus mRNA TSP-4 protein.
X89963_1	0.008084703	unknown function
AW144778	0.007854843	Rattus norvegicus multiple endocrine neoplasia 1 (Men1), mRNA.
NM_019208	0.007826723	

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
219406_Rn	0.007688372	similar to mouse CLST 11240 protein, clone MGC:11725 IMAGE:3967350, mRNA, complete cds.
A1170794	0.007664342	unknown function
CB547931	0.007544952	unknown function
265390_Rn	0.007506835	similar to Mouse DNA sequence from clone RP23-132M9 on chromosome X, complete sequence.
222840_Rn	0.006799741	Rat mRNA for band83, complete cds.
287902_Rn	0.006780544	similar to Mouse, asporin, clone MGC:41375 IMAGE:1365428, mRNA, complete cds.
230838_Rn	0.006544266	similar to unnamed protein product
BF398773	0.006497757	unknown function
CB544919	0.006493518	similar to Protein:NP_060587 WD40 repeat domain 11 protein; WD repeat domain 15 [Homo sapiens]. score=8.275e-65
295324_Rn	0.006476527	similar to Mouse ankyrin repeat-containing SOCS box protein 5 (Asb5) mRNA, complete cds.
208497_Rn	0.00643506	similar to Translation of nuc:AF035526_1 Mus musculus kanadaptin mRNA, complete cds. score=6.115e-20
CB606361	0.006152612	similar to Translation of nuc:AK027583_1 Homo sapiens cDNA FLJ14677 fts, clone NT2RP2004095, unnamed protein product. score=6.259e-177
		Rat MS1 mRNA, complete cds.
296678_Rn	0.005926665	unknown function
208020_Rn	0.005876574	unknown function
CB546554	0.00552919	similar to Mouse, clone IMAGE:3598145, mRNA, partial cds.
203945_Rn	0.005471138	similar to Mouse early quiescence protein-1 mRNA, complete cds.
347349_Rn	0.005333745	unknown function
BI291651	0.004985429	similar to Mouse histidine-rich Ca ²⁺ binding protein mRNA, complete cds.
296737_Rn	0.0044435824	Rattus norvegicus Clusterin (Clu), mRNA.
200268_Rn	0.004369805	similar to Translation of nuc:AX055831_1 Sequence 3 from Patent WO0073459; unnamed protein product. score=5.006e-65
230761_Rn	0.004313925	Rattus norvegicus small muscle protein, X-linked (Smpx), mRNA.
296860_Rn	0.004274834	Rattus norvegicus connective tissue growth factor (Ctgf), mRNA.
298840_Rn	0.00395162	unknown function
AW915945	0.003930409	Rattus norvegicus cholinergic receptor, nicotinic, delta polypeptide (Chrnd), mRNA.
297051_Rn	0.003823021	unknown function
AA819234	0.003735561	similar to Translation of nuc:AB037835_1 Homo sapiens mRNA for KIAA1414 protein, partial cds; Start codon is not identified. fnt14368 cDNA clone for KIAA1414 has a 1-bp insertion at the position 1878 and a 152-bp deletion at the position between 4092 and 4245 of the sequence of KIAA1414.. score=4.604e-37
CB546661	0.003727216	unknown function
BF417300	0.003664897	unknown function

**Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
CB547992	0.003377652	similar to Protein:NP_082427 RIKEN cDNA 2610528A15 [Mus musculus]. score=3.596e-45
296414_Rn	0.003353914	unknown function
NM_012786	0.003310131	Rattus norvegicus Cytochrom c oxidase subunit VIII-H (heart/muscle) (Cox8h), mRNA.
NM_021588	0.003286342	Rattus norvegicus myoglobin (Mb), mRNA.
295880_Rn	0.003014336	UI-R-A0-ad-e-10-0-UI.s1 UI-R-A0 Rattus norvegicus cDNA clone UI-R-A0-ad-e-10-0-UI 3 similar to gb N62943 N62943 yy68h09.s1 Homo sapiens cDNA clone 278753 3, mRNA sequence.
348034_Rn	0.002724736	Rattus norvegicus desmin (Des), mRNA.
207121_Rn	0.002705551	similar to Protein:NP_081115 RIKEN cDNA 1110055E19 [Mus musculus]. score=2.285e-08
296824_Rn	0.002508676	unknown function
221693_Rn	0.002503854	similar to mouse TLH29 protein precursor, clone MGC:25891 IMAGE:4217067, mRNA, complete cds. score=7.92e-55
CB546467	0.00244741	similar to Translation of nuc:BC021222_1 Homo sapiens, clone MGC:12933 IMAGE:4308662, mRNA, complete cds.
AF420214.1	0.002401046	Rattus norvegicus tumor necrosis factor receptor type II mRNA, partial cds.
348232_Rn	0.002400362	Rattus norvegicus tenomodulin (Tnmd), mRNA.
295561_Rn	0.002366005	unknown function
CB544878	0.002258728	unknown function
BF564613	0.002120915	similar to Protein:NP_542439 Bmp2-inducible kinase [Mus musculus]. score=1.561e-142
NM_017149	0.002093591	Rattus norvegicus mesenchyme homeo box 2 (Meox2), mRNA.
NM_080698	0.001854949	Rattus norvegicus fibromodulin (Fmod), mRNA.
229255_Rn	0.001812349	similar to Mouse, clone IMAGE:4988852, mRNA, partial cds.
202946_Rn	0.001802405	similar to Mouse, RIKEN cDNA 1110018M03 gene, clone MGC:29048 IMAGE:3603588, mRNA, complete cds.
293007_Rn	0.001780344	Rattus norvegicus serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (Serpine1), mRNA.
291388_Rn	0.001588115	Rattus norvegicus cartilage link protein 1 (Crtl1), mRNA.
NM_021763	0.001552436	Rattus norvegicus arfaptin 1 (LOC60382), mRNA.
J04628.1	0.001524132	GCGNUC_04628.1; Rattus norvegicus 3-hydroxyiso- butyrate mRNA, 3 end.
NM_138900	0.001494911	Rattus norvegicus complement component 1, s subcomponent (C1s), mRNA.
413271_Rn	0.001423915	similar to Mouse, secreted modular calcium binding protein 2, clone MGC:28521 IMAGE:4191849, mRNA, complete cds.
NM_013104	0.001375015	Rattus norvegicus Insulin-like growth factor binding protein 6 (lgfbpp6), mRNA.
AW142985	0.00125441	unknown function
430014_Rn	0.001182215	Rat troponin-c mRNA.

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
206212_Rn	0.001159988	Rattus norvegicus actinin alpha 2 associated LIM protein (Pdlim3), mRNA.
202503_Rn	0.001151385	Rattus norvegicus histone deacetylase 2 (Hdac2) mRNA, partial cds.
220427_Rn	0.001019677	similar to Mouse, clone IMAGE:3991175, mRNA, partial cds.
201221_Rn	9.85E-04	unknown function
AA818120	8.96E-04	unknown function
220847_Rn	7.93E-04	Rattus norvegicus C4 complement protein mRNA, partial cds.
AA957467	7.87E-04	similar to Translation of nuc:AK003750_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110017116, full insert sequence; putative. score=2.217e-25
CB547949	7.52E-04	similar to Protein:NP_573478 myotubularin related protein 4; FYVE zinc finger phosphatase [Mus musculus]. score=7.888e-93
299009_Rn	7.46E-04	similar to Mouse carboxypeptidase X2 mRNA, complete cds.
258706_Rn	7.15E-04	unknown function
CB546345	6.98E-04	similar to Protein:NP_033996 cadherin 11; OB-cadherin; osteoblast-cadherin [Mus musculus]. score=3.453e-09
282820_Rn	6.92E-04	unknown function
BF415205	5.51E-04	Rat mRNA fragment for cardiac actin.
BM388714	5.33E-04	unknown function
BF523437	5.22E-04	unknown function
CB546505	5.15E-04	similar to Protein:NP_033010 protein tyrosine phosphatase, receptor-type, M [Mus musculus]. score=1.044e-80
222627_Rn	5.02E-04	Rattus norvegicus Troponin I, slow isoform (Tnni1), mRNA.
217038_Rn	4.92E-04	Rattus norvegicus Insulin-like growth factor II (somatomedin A) (Igf2), mRNA.
NM_057191	4.58E-04	Rattus norvegicus sarcomeric muscle protein (Sarcosin), mRNA.
207465_Rn	4.56E-04	similar to Translation of nuc:AK003938_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110027012, full insert sequence; putative. score=7.721e-11
232141_Rn	3.45E-04	similar to cartilage intermediate layer protein
201251_Rn	3.22E-04	similar to Mouse putative potassium-chloride cotransporter-4 (Kcc4) mRNA, complete cds.
200736_Rn	2.01E-04	Rattus norvegicus myosin heavy chain, polypeptide 6, cardiac muscle, alpha (Myh6), mRNA.
AI579422	1.58E-04	unknown function
NM_013220	1.52E-04	Rattus norvegicus ankyrin-like repeat protein (Alrp), mRNA.
295023_Rn	1.43E-04	similar to Translation of nuc:AK003750_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110017116, full insert sequence; putative. score=2.217e-25
AI409191	1.35E-04	unknown function

**Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing
Greater than 2-Fold UP-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
347198_Rn	1.33E-04	similar to Mouse, RIKEN cDNA 2310045A07 gene, clone MGC:41044 IMAGE:1397989, mRNA, complete cds.
229336_Rn	1.20E-04	similar to Mouse collagenous repeat-containing sequence of 26kDa protein (CORS26) mRNA, complete cds.
NM_030868	7.75E-05	Rattus norvegicus NOV protein (Nov), mRNA.
327164_Rn	6.21E-05	similar to Mouse REX-3 mRNA, complete cds.
199437_Rn	5.94E-05	Rattus norvegicus Neuron specific protein PEP-19 (Purkinje cell protein 4) (Pcp4), mRNA.
217522_Rn	5.67E-05	Rattus norvegicus actinin alpha 3 (Actn3), mRNA.
NM_057144	4.43E-05	Rattus norvegicus cysteine-rich protein 3 (Csp3), mRNA.
NM_022499	1.02E-05	Rattus norvegicus Parvalbumin (calcium binding protein) (Pvalb), mRNA.
284707_Rn	8.63E-06	Rattus norvegicus chondromodulin-1 (Chm-1), mRNA.
NM_012929	5.35E-07	Rattus norvegicus Procollagen II alpha 1 (Col2a1), mRNA.
BF560915	4.85E-07	Rattus norvegicus mRNA for collagen alpha 1 type X, partial.

Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing Greater than 2-Fold DOWN-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
297538_Rn	0.04702519	similar to Mouse mRNA for Ky protein (muscle-specific protein).
NM_012589	0.046647282	Rattus norvegicus Interleukin 6 (interferon, beta 2) (Il6), mRNA.
296913_Rn	0.04407278	similar to Mouse myocytic induction/differentiation originator mRNA, complete cds.
BE115700	0.04088808	unknown function
AW918099	0.036855822	
217925_Rn	0.0366667956	similar to Mouse mRNA for M-protein.
292212_Rn	0.033939721	Rattus norvegicus Small inducible gene JE (Scya2), mRNA.
BQ781375	0.033572781	similar to Protein:NP_067483 myozinin; skeletal muscle-specific protein; calcineurin-2 [Mus musculus]. score=7.91e-40
219978_Rn	0.033154467	Rat mRNA for glycerol 3-phosphate dehydrogenase, complete cds.
218360_Rn	0.026208044	similar to Mouse, integrin beta 1 binding protein 2, clone MGC:41122 IMAGE:1346742, mRNA, complete cds.
AF059344.1	0.020868903	Rattus norvegicus titin mRNA, partial cds.
NM_022177	0.017225533	Rattus norvegicus Stromal cell-derived factor 1 (Sdf1), mRNA.
NM_017328	0.016465715	Rattus norvegicus phosphoglycerate mutase 2 (Pgam2), mRNA.
NM_017066	0.016209628	Rattus norvegicus Pleiotrophin (Heparine binding factor, Hbnf, in the mouse) (Ptn), mRNA.
220752_Rn	0.014938742	Rattus norvegicus sodium channel, voltage-gated, type I, beta polypeptide (Scn1b), mRNA.
297410_Rn	0.013684151	Rattus norvegicus ryanodine receptor type 1 (RyR1) mRNA, partial cds.
204921_Rn	0.013543894	similar to Translation of nuc:AY036877_1 Mus musculus actinin alpha 2 (Actn2) mRNA, complete cds. score=6.755e-91
AA900269	0.011681645	similar to gi 1304380 gb U29528 RNU29528 Rattus norvegicus hemoglobin alpha chain gene, complete cds, mRNA sequence.
205248_Rn	0.01159689	similar to sarcoplasmic reticulum glycoprotein
204961_Rn	0.009391028	similar to unnamed protein product
BF550734	0.009282715	unknown function
226731_Rn	0.008615446	similar to Translation of nuc:AK009352_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310015C21, full insert sequence; putative, score=1.161e-42
326761_Rn	0.007156469	similar to Mouse RING-finger protein MURF mRNA, complete cds.
220641_Rn	0.006185176	similar to data source:SPTR, source key:Q99969, evidence:ISS~homolog to RETINOIC ACID RECEPTOR RESPONDER PROTEIN 2 PRECURSOR (TAZAROTENE- INDUCED GENE 2 PROTEIN) (RAR-RESPONSIVE PROTEIN TIG2)~putative
222395_Rn	0.006176233	Rat mRNA for sarcomeric mitochondrial creatine kinase.
BQ194536	0.006085215	similar to Protein:NP_033824 apolipoprotein B editing complex 2 [Mus musculus]. score=7.281e-97

**Table 2: Significant 2-Fold Gene Expression Changes at 11 Days Fracture Healing
Greater than 2-Fold DOWN-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
220080_Rn	0.005352806	similar to Protein:NP_067483 myozinin; skeletal muscle-specific protein; calcineurin-2 [Mus musculus]. score=4.677e-48
200403_Rn	0.003595361	Rat mRNA for atrial myosin light chain 1.
218799_Rn	0.003300111	similar to Mouse mRNA for stretch responsive protein (gene Tims).
200849_Rn	0.00273697	Rattus norvegicus Creatine kinase, muscle form (Ckm), mRNA.
296247_Rn	0.002724828	similar to Mouse cypher1 mRNA, complete cds.
280399_Rn	0.002007331	similar to mouse Ig joining chain, clone MGC:6626 IMAGE:3491646, mRNA, complete cds.
216910_Rn	0.001884423	similar to Protein:NP_033824 apolipoprotein B editing complex 2 [Mus musculus]. score=7.281e-97
BF285291	0.001606058	unknown function
NM_031813	4.80E-04	Rattus norvegicus norvegicus myosin binding protein H (Mybph), mRNA.
CB548063	2.82E-04	similar to Translation of nuc:BC022248_1 Homo sapiens, Similar to Dnaj (Hsp40) homolog, subfamily B, member 12, clone MGC:22187 IMAGE:4771526, mRNA, complete cds. score=2.211e-56

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
293162_Rn	0.049065665	similar to hypothetical protein FLJ13236
344269_Rn	0.049065665	Rattus norvegicus sodium-coupled citrate transporter (NaCT), mRNA.
BQ204885	0.047362652	unknown function
NM_019289	0.047362652	Rattus norvegicus Actin-related protein complex 1b (Arpc1b), mRNA.
296678_Rn	0.047315244	Rat MS1 mRNA, complete cds.
BQ208015	0.046880281	similar to Protein:NP_081121 peptidyl/prolyl isomerase (cyclophilin)-like 1 [Mus musculus]. score=9.871e-82
230838_Rn	0.046366731	similar to unnamed protein product
CB547489	0.04572215	similar to Translation of nuc:AB067489_1 Homo sapiens mRNA for KIAA1902 protein, partial cds; Start codon is not identified.. score=3.858e-51
222395_Rn	0.045331181	Rat mRNA for sarcomeric mitochondrial creatine kinase.
CB544919	0.045211033	similar to Protein:NP_060587 WD40 repeat domain 11 protein; WD repeat domain 15 [Homo sapiens]. score=8.275e-65
203358_Rn	0.045211033	Rat c-fos mRNA.
CB546554	0.045200028	unknown function
217893_Rn	0.045200028	Rattus norvegicus heat shock 70kD protein 5 (Hspa5), mRNA.
222534_Rn	0.045072793	similar to Mouse, clone IMAGE:3583970, mRNA, partial cds.
BF398773	0.043516823	unknown function
218904_Rn	0.043516823	similar to KIAA0782 protein
AA858954	0.043073552	unknown function
221755_Rn	0.043073552	unknown function
203945_Rn	0.042985576	similar to Mouse, clone IMAGE:3598145, mRNA, partial cds.
208250_Rn	0.042497156	Rattus norvegicus interferon gamma receptor (Ifngr), mRNA.
219309_Rn	0.042231739	Rat heme oxygenase gene, complete cds.
AA819234	0.041928152	unknown function
BE107038	0.041928152	unknown function
217925_Rn	0.041287368	similar to Mouse mRNA for M-protein.
NM_012587	0.041287368	Rattus norvegicus integrin binding sialoprotein (Ibsp), mRNA.
287902_Rn	0.040389711	similar to Mouse, asporin, clone MGC:41375 IMAGE:1365428, mRNA, complete cds.
298983_Rn	0.039487435	similar to myopalladin
219058_Rn	0.039020125	Rattus norvegicus S100 calcium-binding protein A9 (calgranulin B) (S100a9), mRNA.
226731_Rn	0.038435721	similar to Translation of nuc:AK009352_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310015C21, full insert sequence; putative. score=1.161e-42

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
205248_Rn	0.037833766	similar to sarcoplasmic reticulum glycoprotein
U57362_1	0.037810091	Rattus norvegicus collagen XII alpha 1 (Col12a1) mRNA, partial cds.
203776_Rn	0.037459117	Rattus norvegicus glyccoprotein (transmembrane) nmb (Gpnmb), mRNA.
220641_Rn	0.035760314	similar to data source:SPTR, source key:Q99969, evidence:iSS~homolog to RETINOIC ACID RECEPTOR RESPONDER PROTEIN 2 PRECURSOR (TAZAROTENE- INDUCED GENE 2 PROTEIN) (RAR-RESPONSIVE PROTEIN TG2)~putative
AI411057	0.034915752	unknown function
NM_012786	0.034177803	Rattus norvegicus Cytochrome c oxidase subunit VIII-H (heart/muscle) (Cox8h), mRNA.
298840_Rn	0.034177803	Rattus norvegicus connective tissue growth factor (Ctgf), mRNA.
298460_Rn	0.034081546	Rattus norvegicus Early growth response 1 (Egr1), mRNA.
BQ209028	0.033524821	unknown function
322818_Rn	0.033524821	similar to Mouse, clone IMAGE:3489640, mRNA.
215841_Rn	0.033446149	similar to Mouse DNA sequence from clone RP23-406B13 on chromosome 4, complete sequence.
202815_Rn	0.032914575	similar to Mouse, RIKEN cDNA 1300011C24 gene, clone MGC:7169 IMAGE:3257323, mRNA, complete cds.
BF417300	0.032885657	unknown function
BF558699	0.032580888	Rattus norvegicus Clusterin (Clu), mRNA.
200268_Rn	0.032237305	similar to Protein:NP_080731 RIKEN cDNA 2510049119 gene [Mus musculus]. score=5.034e-32
221395_Rn	0.030997999	similar to Translation of nuc:AK027583_1 Homo sapiens cDNA FLJ14677 fis, clone NT2RP2004095; unnamed protein product. score=6.259e-177
CB606361	0.030997999	Rattus norvegicus Benzodiazepin receptor (peripheral) (Bzrp), mRNA.
222867_Rn	0.030997999	Rattus norvegicus smooth muscle cell LIM protein (SmLIM) mRNA, complete cds.
206584_Rn	0.030997999	unknown function
BI291651	0.029780122	Rattus norvegicus high-affinity immunoglobulin gamma Fc receptor I mRNA, partial cds.
BQ189985	0.029774468	Rattus norvegicus cysteine rich protein 1 (Csrp1), mRNA.
NM_017148	0.029727025	similar to Translation of nuc:AY036877_1 Mus musculus actinin alpha 2 (Actn2) mRNA, complete cds. score=6.755e-91
204921_Rn	0.028954673	Rattus norvegicus Tumor necrosis factor receptor (Tnfrf1), mRNA.
NM_013091	0.028352338	UI-R-A0-ad-e-10-0-UI.s1 UI-R-A0 Rattus norvegicus cDNA clone UI-R-A0-ad-e-10-0-UI 3 similar to gb N62943 N62943 yy68h09.s1 Homo sapiens cDNA clone 278753 3, mRNA sequence.
295880_Rn	0.028352338	unknown function
AW918099	0.028352338	Rattus norvegicus phosphoglycerate mutase 2 (Pgam2), mRNA.
NM_017328	0.027215663	

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
CB546661	0.026862147	similar to Translation of nuc:AB037835_1 Homo sapiens mRNA for KIAA1414 protein, partial cds; Start codon is not identified. fh14368 cDNA clone for KIAA1414 has a 1-bp insertion at the position 1878 and a 152-bp deletion at the position between 4092 and 4245 of the sequence of KIAA1414.. score=4.604e-37
218553_Rn	0.026697054	similar to Protein:NP_031447 adenylosuccinate synthetase 1, muscle [Mus musculus]. score=5.795e-85
AW915945	0.026697054	unknown function
348034_Rn	0.026693981	Rattus norvegicus desmin (Des), mRNA.
413406_Rn	0.026693981	UI-R-A1-eo-f-12-0-Ui.s1 UI-R-A1 Rattus norvegicus cDNA clone UI-R-A1-eo-f-12-0-Ui 3 similar to gi 2918669 dbj C86712 C86712 Mus musculus fertilized egg cDNA 3-end sequence, clone J0231G01, mRNA sequence.
200661_Rn	0.026625164	similar to Mouse, clone MGC:6469 IMAGE:2631779, mRNA, complete cds.
NM_134334	0.025034746	Rattus norvegicus cathepsin D (Ctsd), mRNA.
U75926_1	0.025034746	Rattus norvegicus cytochrome oxidase subunit VIIa mRNA, 5 untranslated region, partial sequence.
292212_Rn	0.024852451	Rattus norvegicus Small inducible gene JE (Scya2), mRNA.
347349_Rn	0.023998962	similar to Mouse early quiescence protein-1 mRNA, complete cds.
201560_Rn	0.022807921	similar to Translation of nuc:AK002214_1 Mus musculus adult male kidney cDNA, RIKEN full-length enriched library, clone:0610005A19, full insert sequence; putative. score=1.142e-28
AA858962	0.022807921	Rat retinol-binding protein (RBP) mRNA, partial cds.
BQ200384	0.022807921	unknown function
CB547992	0.022807921	similar to Protein:NP_082427 RIKEN cDNA 2610528A15 [Mus musculus]. score=3.596e-45
CA509950	0.022323819	Rattus sp. 3 UTR.
NM_022510	0.022323819	Rattus norvegicus ribosomal protein L4 (Rpl4), mRNA.
199948_Rn	0.022323819	Rattus norvegicus Farnesyl diphosphate synthase (Fdps), mRNA.
222544_Rn	0.022211891	Rattus norvegicus Cholesterol esterase (β pancreatic) (Lipa), mRNA.
BQ210920	0.021803968	unknown function
BE105985	0.021492965	unknown function
222629_Rn	0.019689063	Rattus norvegicus embigin (Emb), mRNA.
AA858647	0.019409652	unknown function
NM_017149	0.019112443	Rattus norvegicus mesenchyme homeo box 2 (Meox2), mRNA.
221213_Rn	0.019112443	Rattus norvegicus Syndecan 1 (Sdc1), mRNA.
204961_Rn	0.017996057	similar to unnamed protein product
AW525201	0.017996057	unknown function
296737_Rn	0.017442419	similar to Mouse histidine-rich Ca ²⁺ binding protein mRNA, complete cds.
297051_Rn	0.016703667	Rattus norvegicus cholinergic receptor, nicotinic, delta polypeptide (Chrnd), mRNA.

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
230761_Rn	0.016479583	similar to Translation of nuc:AX055831_1 Sequence 3 from Patent WO0073459; unnamed protein product. score=5.006e-65
NM_013026	0.016103594	Rattus norvegicus Syndecan (Synd1), mRNA.
201953_Rn	0.01598859	similar to Mouse ElA (Serpib1) mRNA, complete cds.
206738_Rn	0.01598859	Rat metallothionein-2 and metallothionein-1 genes, complete cds.
295561_Rn	0.015844812	unknown function
NM_080698	0.015681369	Rattus norvegicus fibromodulin (Fmod), mRNA.
207121_Rn	0.014645637	similar to Protein:NP_081115 RIKEN cDNA 1110055E19 [Mus musculus]. score=2.285e-08
CB545131	0.014645637	similar to Protein:NP_055392 bromodomain containing protein 1; BR140-like gene [Homo sapiens]. score=7.563e-63
BQ194536	0.014645637	similar to Protein:NP_033824 apolipoprotein B editing complex 2 [Mus musculus]. score=7.281e-97
219818_Rn	0.014447432	similar to Mouse spermidine/spermine N1-acetyltransferase (SSAT) mRNA, complete cds.
AA859114	0.014447432	similar to Protein:NP_080712 RIKEN cDNA 1810054O13 [Mus musculus]. score=6.377e-40
CB546467	0.014338782	similar to Translation of nuc:BC021222_1 Homo sapiens, clone MGC:12933 IMAGE:4308662, mRNA, complete cds. score=7.92e-55
AF420214_1	0.014338782	Rattus norvegicus tumor necrosis factor receptor type II mRNA, partial cds.
221693_Rn	0.014318903	similar to mouse TLH29 protein precursor, clone MGC:25891 IMAGE:4217067, mRNA, complete cds.
202946_Rn	0.013828435	similar to Mouse, RIKEN cDNA 1110018M03 gene, clone MGC:29048 IMAGE:3603588, mRNA, complete cds.
295729_Rn	0.013579155	Rat thymocyte L-Ca (leukocyte common antigen) mRNA, 3' flank.
220080_Rn	0.0133583	similar to Protein:NP_067483 myozentin; skeletal muscle-specific protein; calcineurin-2 [Mus musculus]. score=4.677e-48
199927_Rn	0.012716027	Rattus norvegicus H3 histone, family 3B (H3f3b), mRNA.
296824_Rn	0.012547921	unknown function
NM_021588	0.012547921	Rattus norvegicus myoglobin (Mb), mRNA.
296860_Rn	0.012538196	Rattus norvegicus small muscle protein, X-linked (Smox), mRNA.
200663_Rn	0.012538196	Rattus norvegicus vimentin (Vim), mRNA.
BF564613	0.012538196	similar to Protein:NP_542439 Bmp2-inducible kinase [Mus musculus]. score=1.561e-142
229255_Rn	0.012538196	similar to Mouse, clone IMAGE:4988852, mRNA, partial cds.
NM_138900	0.012538196	Rattus norvegicus complement component 1, s subcomponent (C1s), mRNA.
231072_Rn	0.012538196	similar to Mouse, clone IMAGE:3589736, mRNA, partial cds.
292104_Rn	0.012277109	similar to Translation of nuc:BC032959_1 Mus musculus, triggering receptor expressed on myeloid cells 2a, clone MGC:40999 IMAGE:1348066, mRNA, complete cds. score=2.498e-69

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
200403_Rn	0.012277109	Rat mRNA for atrial myosin light chain 1.
221153_Rn	0.011881815	Rattus norvegicus mRNA for cathepsin Y, partial cds.
CB548315	0.011881815	similar to Protein:NP_084515 ATP-binding cassette, subfamily G, member 3 [Mus musculus]. score=6.816e-62
385033_Rn	0.011794992	Rattus norvegicus high mobility group box 2 (Hmgb2), mRNA.
206075_Rn	0.011636887	similar to mouse annexin A8, clone MGC:13875 IMAGE:4013266, mRNA, complete cds.
NM_017066	0.011403199	Rattus norvegicus Pleiotrophin (Heparine binding factor, Hbnf, in the mouse) (Ptn), mRNA.
296381_Rn	0.011403199	similar to RAB5 interacting protein 3
NM_021763	0.011306492	Rattus norvegicus arafaptin 1 (LOC60382), mRNA.
CB547219	0.011202141	unknown function
AA900381	0.010708936	unknown function
292230_Rn	0.010664541	Rattus norvegicus dual specificity phosphatase 6 (Dusp6), mRNA.
NM_031813	0.010108819	Rattus norvegicus norvegicus myosin binding protein H (Mybph), mRNA.
NM_138828	0.009795748	Rattus norvegicus Apolipoprotein E, (Apoe), mRNA.
206212_Rn	0.009795748	Rattus norvegicus actinin alpha 2 associated LIM protein (Pdlim3), mRNA.
BQ781375	0.009540745	similar to Protein:NP_067483 myozentin; skeletal muscle-specific protein; calcineurin-2 [Mus musculus]. score=7.91e-40
NM_017055	0.009481938	Rattus norvegicus Transferrin (Tf), mRNA.
413271_Rn	0.009251402	similar to Mouse, secreted modular calcium binding protein 2, clone MGC:28521 IMAGE:4191849, mRNA, complete cds.
273885_Rn	0.009137271	Rattus norvegicus complement component 5, receptor 1 (C5r1), mRNA.
298020_Rn	0.00894185	similar to Mouse homeobox gene Prx2 mRNA.
NM_013156	0.00894185	Rattus norvegicus Cathepsin L (Ctsl), mRNA.
280399_Rn	0.00851745	similar to mouse Ig joining chain, clone MGC:6626 IMAGE:3491646, mRNA, complete cds.
202503_Rn	0.00846734	Rattus norvegicus histone deacetylase 2 (Hdac2) mRNA, partial cds.
NM_013104	0.008252958	Rattus norvegicus Insulin-like growth factor binding protein 6 (Igfbp6), mRNA.
AW142985	0.008252958	unknown function
J04628.1	0.007983283	GCGNUC:J04628.1; Rattus norvegicus 3-hydroxyiso- butyrate mRNA, 3 end.
217733_Rn	0.007983283	similar to Mouse, RIKEN cDNA 1300019103 gene, clone MGC:6625 IMAGE:3491528, mRNA, complete cds.
208320_Rn	0.007816396	similar to evidence:NAS~hypothetical protein~putative
296247_Rn	0.007816396	similar to Mouse cypher1 mRNA, complete cds.
348232_Rn	0.007816396	Rattus norvegicus tenomodulin (Tnmd), mRNA.

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_053843	0.007801117	Rattus norvegicus Fc receptor, IgG, low affinity III (Fcgr3), mRNA.
223588_Rn	0.007578489	Rattus norvegicus macrophage galactose N-acetyl-galactosamine specific lectin (Mgl), mRNA.
294206_Rn	0.007532748	similar to Mouse strain C57BL/6J basigin mRNA, complete cds.
CB606336	0.006914106	unknown function
343323_Rn	0.006486278	similar to Mouse FKBP65 binding protein mRNA, complete cds.
208234_Rn	0.005333962	Rattus norvegicus coronin, actin binding protein 1A (Coro1a), mRNA.
216910_Rn	0.005333962	similar to Protein:NP_033824 apolipoprotein B editing complex 2 [Mus musculus]. score=7.281e-97
293007_Rn	0.005084269	Rattus norvegicus serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (Serpine1), mRNA.
220847_Rn	0.005084269	Rattus norvegicus C4 complement protein mRNA, partial cds.
220427_Rn	0.005084269	similar to Mouse, clone IMAGE:3991175, mRNA, partial cds.
CB544878	0.005084269	unknown function
201221_Rn	0.004667983	unknown function
282820_Rn	0.00436441	unknown function
CB547949	0.00436441	similar to Protein:NP_573478 myotubularin related protein 4; FYVE zinc finger phosphatase [Mus musculus]. score=7.888e-93
BF285291	0.00436441	unknown function
200849_Rn	0.004166174	Rattus norvegicus Creatine kinase, muscle form (Ckm), mRNA.
CB546345	0.004130372	similar to Protein:NP_033996 cadherin 11; OB-cadherin; osteoblast-cadherin [Mus musculus]. score=3.453e-09
AA818120	0.003743649	unknown function
NM_131914	0.003731393	Rattus norvegicus caveolin 2 (Cav2), mRNA.
BF415205	0.003731393	Rat mRNA fragment for cardiac actin.
NM_057191	0.0033447402	Rattus norvegicus sarcomeric muscle protein (Sarcosin), mRNA.
BF523437	0.0033300256	unknown function
299009_Rn	0.003300256	similar to Mouse carboxypeptidase X2 mRNA, complete cds.
296414_Rn	0.003300256	unknown function
CB546505	0.003300256	similar to Protein:NP_033010 protein tyrosine phosphatase, receptor-type, M [Mus musculus]. score=1.044e-80
208422_Rn	0.002529398	Rattus norvegicus alanyl (membrane) aminopeptidase (Anpep), mRNA.
NM_033539	0.002032439	Rattus norvegicus eukaryotic translation elongation factor 1 alpha 2 (Eef1a2), mRNA.
BM388714	0.0018133	unknown function
AW526982	0.001705411	unknown function

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
CB548041	0.001705411	similar to Protein:NP_663384 similar to cutlet [Mus musculus]. score=1.492e-20
201251_Rn	0.001635916	similar to Mouse putative potassium-chloride cotransporter-4 (Kcc4) mRNA, complete cds.
AA957467	0.001434298	similar to Translation of nuc:AK003750_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110017116, full insert sequence; putative. score=2.217e-25
222627_Rn	0.001395462	Rattus norvegicus Troponin I, slow isoform (Tnni1), mRNA.
412899_Rn	0.001347668	similar to Mouse Strain C57BL6/J Chromosome X BAC, RP23-64A09, Complete Sequence, complete sequence.
CB548063	0.001266003	similar to Translation of nuc:BC022248_1 Homo sapiens, Similar to DnaJ (Hsp40) homolog, subfamily B, member 12, clone MGC:22187 IMAGE:4771526, mRNA, complete cds. score=2.211e-56
217038_Rn	0.001266003	Rattus norvegicus Insulin-like growth factor II (somatomedin A) (Igf2), mRNA.
NM_022226	0.001260916	Rattus norvegicus protease, cysteine, 1 (legumain) (Prsc1), mRNA.
232141_Rn	0.001260916	similar to cartilage intermediate layer protein
BQ205045	8.84E-04	unknown function
NM_012771	8.60E-04	Rattus norvegicus Lysosome (Lyz), mRNA.
207465_Rn	8.06E-04	similar to Translation of nuc:AK003938_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110027012, full insert sequence; putative. score=7.721e-11
AI579422	8.03E-04	unknown function
215832_Rn	6.87E-04	similar to Mouse, RIKEN cDNA 3110037K17 gene, clone MGC:41407 IMAGE:1496162, mRNA, complete cds.
NM_013220	6.74E-04	Rattus norvegicus ankyrin-like repeat protein (Alrp), mRNA.
347198_Rn	4.84E-04	similar to Mouse, RIKEN cDNA 2310045A07 gene, clone MGC:41044 IMAGE:1397989, mRNA, complete cds.
258706_Rn	4.84E-04	unknown function
229336_Rn	4.84E-04	similar to Mouse collagenous repeat-containing sequence of 26kDa protein (CORS26) mRNA, complete cds.
AI409191	4.84E-04	unknown function
200736_Rn	4.27E-04	Rattus norvegicus myosin heavy chain, polypeptide 6, cardiac muscle, alpha (Myh6), mRNA.
291388_Rn	2.87E-04	Rattus norvegicus cartilage link protein 1 (Crtl1), mRNA.
327164_Rn	2.87E-04	similar to Mouse REX-3 mRNA, complete cds.
295023_Rn	2.87E-04	similar to Translation of nuc:AK003750_1 Mus musculus 18 days embryo cDNA, RIKEN full-length enriched library, clone:1110017116, full insert sequence; putative. score=2.217e-25
NM_030868	2.87E-04	Rattus norvegicus NOV protein (Nov), mRNA.
217522_Rn	2.87E-04	Rattus norvegicus actinin alpha 3 (Actn3), mRNA.

Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing Greater than 2-Fold UP-regulated

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
NM_057144	2.49E-04	Rattus norvegicus cysteine-rich protein 3 (Csrp3), mRNA.
199437_Rn	2.39E-04	Rattus norvegicus Neuron specific protein PEP-19 (Purkinje cell protein 4) (Pcp4), mRNA.
NM_022499	2.19E-05	Rattus norvegicus Parvalbumin (calcium binding protein) (Pvalb), mRNA.
284707_Rn	5.00E-07	Rattus norvegicus chondromodulin-1 (Chm-1), mRNA.
NM_012929	8.32E-08	Rattus norvegicus Procollagen II alpha 1 (Col2a1), mRNA.
BF560915	8.32E-08	Rattus norvegicus mRNA for collagen alpha 1 type X, partial.

**Table 3: Significant 2-Fold Gene Expression Changes Between 3 Days and 11 Days Fracture Healing
Greater than 2-Fold DOWN-regulated**

<u>Gene ID</u>	<u>P-value</u>	<u>Gene Function</u>
200837_Rn	0.031732148	Rattus norvegicus defensin NP-2 precursor (LOC286995), mRNA.

Table 3: Significant Gene Expression Changes Between 3 Days and 11 Days Fracture Healing UP or DOWN-regulated LESS than 2-Fold

Gene ID	P-value	Gene Function
NM_019289	0.047362652	Rattus norvegicus Actin-related protein complex 1b (Arpc1b), mRNA.
230838_Rn	0.046366731	similar to unnamed protein product, AI105049
222395_Rn	0.045331181	Rat mRNA for sarcomeric mitochondrial creatine kinase.
BF398773	0.043516823	unknown function
AA858954	0.043073552	unknown function
203945_Rn	0.042985576	similar to Mouse, clone IMAGE:3598145, mRNA, partial cds.
219309_Rn	0.042231739	Rat heme oxygenase gene, complete cds.
226731_Rn	0.038435721	similar to Translation of nuc:AK009352_1 Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310015C21, full insert sequence; putative. score=1.161e-42
230776_Rn	0.037459117	Rattus norvegicus glycoprotein (transmembrane) nmb (Gpnmb), mRNA. similar to data source:SPTR, source key:Q99969, evidence:ISS-homolog to RETINOIC ACID RECEPTOR RESPONDER PROTEIN 2 PRECURSOR (TAZAROTENE- INDUCED GENE 2 PROTEIN) (RAR-RESPONSIVE PROTEIN TIG2)~putative
220641_Rn	0.035760314	unknown function
AI411057	0.034915752	Cytochrome b-245 light chain, Critical component of the membrane-bound oxidase of phagocytes that generates superoxide
BF558699	0.032580888	Rattus norvegicus high mobility group box 2 (Hmgb2), mRNA.
385033_Rn	0.011794992	Rattus norvegicus Transferrin (Tf), mRNA.
NM_017055	0.009481938	